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PARASITOLOGY

EDITED BY

GEORGE H. F. NUTTALL, F.R.S.

QUICK PROFESSOR OF BIOLOGY IN THE UNIVERSITY OF CAMBRIDGE

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VOLUME XII JANUARY, 1920 No. 1

ON COLORATION IN TICKS. II.

BY GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With Plates I and II.)

I. COLORATION IN LIVING TICKS.

IN a previous note on coloration in ticks (iv. 1913, *Parasitology*, VI. 49–51, Pl. VII), reference was made to the appearances observed in living and dead specimens, the differences were indicated by coloured figures of *Amblyomma variegatum* (Fabricius) and *A. splendidum* Giebel, and the hope was expressed that future authors would, when practicable, describe the coloration as it is seen by daylight in living specimens.

As a further contribution to the subject, I herewith offer two coloured plates reproducing water colour drawings from living ticks raised by me in Cambridge between 1912 and 1915, the plates having been prepared for incorporation in our *Monograph of the Ixodoidea*. Camera lucida drawings were made by me as the groundwork for these figures all of which were lithographed by the late Mr Edwin Wilson. The latter painted the specimens under my direction, excepting one (Pl. I, fig. 4) which was drawn and coloured by me.

Amblyomma hebraeum Koch. Plate I, figs. 1 and 2 represent living specimens (N. 1732, ♂ and ♀) as they appeared 5. ii. 1914, *i.e.* two months after ecdysis and prior to feeding. The fasting ticks retain this appearance for months. Pl. I, fig. 4 represents the scutum of a ♂ of the same lot after it had remained anchored upon a ram's scrotum for 77 days; it shows that a remarkable change in coloration may occur in ticks of this species that sojourn long upon the host. After 141 days upon the ram the colours in other specimens were found to have become even more intensified, especially the red areas at the sides. I referred but incidentally to this colour change in a previous paper (*Parasitology*, XI. 395, ♂ 5). For an account of the biology of this species, the carrier of Heartwater in Africa, see *Ibid.* VII. 409–419.

Amblyomma gemma Dönitz. Plate I, figs. 5 and 3 represent the scutums of (N. 3016) living unfed ♂ and ♀ specimens as seen on 4. ix. 1914, about two weeks after ecdysis, the specimens having been received from East Africa.

If we compare the scutums of these two species when alive, or when dead for weeks or years and dried or preserved in 70 per cent. alcohol, we may note the following differences (omitting finer details):

Colour changes in the Scutum.

	Male			Female
	Greater part of pale area	Lateral and marginal pale areas, scapulae	Festoons where pale	Greater part of pale area
In **A. hebraeum**				
Young, unfed, living	Pale violet	Ochre	Pale yellow	Pale yellow
Ditto, dead, dry	Pale yellow to pale yellowish green	Pale ochre	Pale yellow	Dirty yellow
Old, fed, living	Pale green	Red	Bright yellow	—
Old, fed, dead, dry	Pale green	Red*	Bright yellow	—
Ditto in alcohol (old or young)	Deep violet with green or coppery sheen at borders	Dark copper with green sheen	Copper with green sheen	Pink with violet gold and green sheen
In **A. gemma**				
Young, unfed, living	Salmon, fine border of yellow	Yellow	Pale yellow	Orange, middle violet, posterior margin pale violet
Ditto, dead, dry	Pale orange yellow	Pale orange yellow	Pale yellow	Pale yellow
Ditto in alcohol (old or young)	Copper with blue and green sheen	Copper with blue and green sheen	Pale copper	Copper with gold and green sheen at borders

* A few specimens had bleached somewhat after 5 years.

The foregoing table serves to illustrate how very differently the ticks' colours appear according to the conditions in which they are examined. In all of the alcoholic specimens the colours are metallic, *i.e.* when viewed by reflected light the coloured parts have the appearance of burnished metal. As stated in my previous paper (*loc. cit.* p. 50), when dried specimens are placed in alcohol the colours become metallic, and vice versa. I do not know of any ticks that show metallic colours when alive.

Dermacentor.

In the species of this genus herein depicted, metallic colours are absent in alcoholic specimens, therefore the colour changes observed in living and variously preserved examples are not so material as in most of the other ornate ticks. In dead specimens, the faint greenish tint seen in the illustration of *D. venustus* may disappear and the darker areas may become blackish in *D. reticulatus niveus.*

Dermacentor reticulatus niveus Neumann. Plate II, figs. 4 and 5 represent (N. 2156) a ♂ and ♀ received in May 1913 from Pina, Spain. I have described the biology of this tick elsewhere, having raised the progeny of the pair here figured (see iii. 1915, *Parasitology*, VII. 421–425).

Dermacentor venustus Banks. Plate II, figs. 1 and 2 represent the ♂ and ♀ of this North American species which is the carrier of Rocky Mountain Fever to man. The specimens figured (N. 1731) were received in June 1912 from Ottawa, Canada. A description of the biology of this species will be found elsewhere (*loc. cit.* pp. 425–430).

Dermacentor variabilis Hunter and Hooker, of which the ♂ is illustrated in Plate II, fig. 3, is likewise an American species. The ornamentation is usually more widely distributed upon the scutum than figured. The illustration is from a solitary adult that I succeeded in raising in Cambridge from (N. 1824) a lot of larvae received in October 1912 from Dr S. Hadwen, the ticks being the offspring of females found 24. vi. 1912 on a horse at Glenora, Manitoba, Canada.

The larvae were placed on a rabbit on 11. x. 1912, in Cambridge. Only 5 gorged larvae were collected, 2 abandoned the host after 4 days, 3 after 5, 7 and 9 days respectively, the rabbit being kept in a room at 15° C. Metamorphosis from larva to nymph took place in 11, 11, 12, 13, 13 days respectively at 21° C. The 5 nymphs were placed upon a rabbit 17–23 days after emergence, the rabbit being kept at 13° C. Only one gorged nymph was recovered after 21 days; it was maintained at 24° C., and after 14 days a ♂ emerged. This ♂ survived unfed for 57 days at 22° C.

A fuller account of the biology of *D. variabilis* has been given by Hadwen (i. 1913, *Parasitology*, v. 234–237).

II. ON THE EFFECT OF TREATMENT WITH CAUSTIC POTASH UPON THE PIGMENTED PARTS OF ORNATE IXODIDAE.

Whilst examining some specimens of *Dermacentor occidentalis* that had been treated with caustic potash and mounted by me in balsam in 1879, I was struck by the circumstance that the creamy white pattern was clearly visible when the transparent specimens were viewed by *reflected* light, whereas specimens of other ornate ticks, similarly prepared, did not show this appearance. After having examined the balsam-mounted material that was available in my cabinet, I selected a series of specimens of ornate ticks from my collection of alcohol preserved material, treated them all alike with caustic potash, and mounted them in balsam.

The ticks of the new series (5. vi. 1915) were placed in 10 per cent. caustic potash solution at ca. 20° C. for 48 hours, then transferred to water for 24 hours, after which they were pricked and pressed between layers of filter paper, cleaned, and placed for 2 days in acidulated (H_2SO_4) water and passed through graded alcohols and clove oil prior to being mounted in balsam.

The examination of the cleared and mounted specimens of the males of different species gave the following results: observations on older material (indicated by o in the list) being included:

Dermacentor	Creamy pattern on scutum, etc. (× = persists, ○ = absent)
albipictus (Packard)	× at scapulae, basis capituli, palps, legs
auratus Supino	× at scapulae, basis capituli, legs
o *occidentalis* Marx	× × complete
reticulatus (Fabricius)	× × complete in 1 ♂
" "	× at scapulae, legs, basis capituli, palps in 1 ♂
o *venustus* Banks	× × complete in 3 ♂♂
rhinocerotis (de Geer)	○

	Creamy pattern on scutum, etc. (× = persists, ○ = absent)
Rhipicephalus	
dux Dönitz	× traces where ornate
maculatus Neumann	× at posterior spots, slightly at centre
pulchellus (Gerstäcker)	× at scapular and antero-lateral spots
Amblyomma	
cajennense Koch	× very slight at light parts
cohaerens Dönitz	○
o *cooperi* Nuttall and Warburton	○
o *decoratum* Koch	○
" "	× very slight at scapulae and marginal spots
dissimile Koch	× scapular spots
gemma Dönitz	○
o *hebraeum* Koch	○
o *hirtum* Neumann	○
marmoreum Koch	× very slight at scapulae
petersi Karsch	○
pomposum Dönitz	× middle field, antero-lateral fields, trace in first festoons
o *splendidum* Dönitz	○
testudinarium Koch	× faintly in middle field and basis capituli
o *uncatum* Nuttall and Warburton	× where spotted posteriorly
o *variegatum* (Fabricius)	× at emargination in middle field and light scapular spots
Aponomma	
decorosum L. Koch	○
exornatum Koch	× at scapulae
o *gervaisi* (Lucas)	○ or × traces in lateral fields (2 ♂♂)
o *gervaisi* var. *lucasi* Warburton	○ in 2 ♂♂
Hyalomma	
o *monstrosum* Nuttall and Warburton	○

In addition to the foregoing, I have examined the whole of Dr C. W. Stiles' caustic-treated balsam-mounted *Dermacentor* material, comprising 58 adults, all of which shows the creamy white coloration previously referred to when viewed by reflected light. Stiles' material comprises specimens labelled *Dermacentor electus* Koch (2 ♂ 1 ♀), *D. occidentalis* Marx (1 ♂ 3 ♀), *D. andersoni* Stiles (9 ♂ 39 ♀) and *Dermacentor* sp. (2 ♂ 1 ♀), all of which are North American species.

The foregoing records show that after caustic potash treatment the creamy markings only appear to persist clearly and completely in certain members of the genera *Dermacentor* and *Rhipicephalus*. Under a high magnification the chitin in the region of the creamy markings appears finely granular as if it included particulate matter. *Dermacentor rhinocerotis*, which yields different results to the other members of its genus that have been examined, bears dull yellowish spots on its scutum in dried specimens, whereas in well-preserved alcoholic specimens the spots have a yellow or pale pink metallic lustre; neither before or after caustic treatment does the species show a creamy ornamentation. The three species of *Rhipicephalus* examined are the only ones that are at present known to be ornate.

In the remaining genera, when specimens are treated with caustic, all

remains of pattern either disappear or but traces remain in certain regions perhaps partly because these are less accessible to contact with the caustic.

On the other hand, where the scutum is dark brown or black, as in certain thicker and well-defined portions where "dark markings" are referred to in descriptions, these markings persist after caustic treatment. It is interesting to note that the bright orange spot occupying the centre of the scutum in *Amblyomma splendidum* ♂ (illustrated by me in *Parasitology*, VI. Pl. VII, figs. 5 and 6) appears to offer an exception in that it corresponds to a dark spot in caustic-treated specimens, whilst no similarly situated dark spot is observable in the scutums of the closely allied species *A. hebraeum*, *A. cohaerens*, etc.

SUMMARY AND CONCLUSIONS.

The coloration of *Amblyomma hebraeum*, *A. gemma*, *Dermacentor venustus*, *D. variabilis* and *D. reticulatus niveus*, as seen in living examples, is depicted for the first time, and the desirability of recording the colours of ornate ticks when alive is indicated.

A remarkable change in colour in living specimens of *A. hebraeum* is described and figured, this change taking place after a prolonged sojourn (74–141 days) upon the host. Such a colour change has not hitherto been observed in ticks. The difference in colour is seen in dead dried specimens but is not appreciable in those preserved in alcohol.

Since the immature stages of ornate ticks are inornate, and the colours change in adults of some species during prolonged periods of parasitism, it is evident that the coloration in adults must depend upon the accumulated products of metabolism beneath the chitinous exoskeleton, the regional distribution of colour depending upon special metabolic functions taking place in corresponding parts of the tick. The ornamental colour-producing layer can be scraped away from the underside of the scutum in most ornate ticks and it is removable from such ticks by the use of caustic potash.

The whitish or creamy coloration that is so characteristic of most species of *Dermacentor* and the three ornate species of *Rhipicephalus* that are known to science, appears on the other hand to depend largely upon inclusions or structural changes within the chitin itself, whence the persistence of the creaminess seen by reflected light in the caustic-treated ticks. Similar, but less distinct, appearances may be seen in ticks belonging to other genera. *Dermacentor rhinocerotis*, which does not exhibit creamy coloration but only dull yellowish spots when dry, is totally decolorized by caustic potash, whilst contrary to most species of its genus it shows metallic coloration in well-preserved specimens in alcohol.

The examination of caustic-treated specimens was carried out upon 31 species of ornate ticks, *i.e. Dermacentor* (8), *Rhipicephalus* (3), *Amblyomma* (15), *Aponomma* (4) and *Hyalomma* (1 species).

The coloration and creamy ornamentation, herein distinguished, appear

to be confined to the thinner portions of the exoskeleton. That the characteristic dark markings correspond to thicker and more darkly chitinised portions of the scutum is demonstrable by dissections, caustic-treated, or sectioned specimens; this being especially evident for instance in *Amblyomma*. The orange spot on the scutum of *A. splendidum* offers an exception.

The optical and chemical study of coloration in ticks deserves further investigation.

DESCRIPTION OF PLATES I AND II.

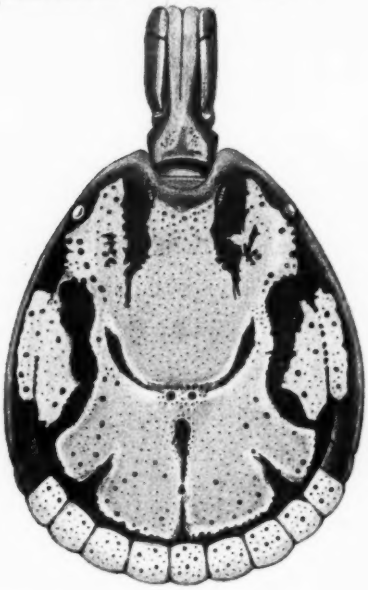
All of the specimens were viewed by daylight and magnified about × 12 under a Zeiss binocula Dissecting Microscope.

PLATE I.

Fig. 1. *Amblyomma hebraeum* Koch. Living unfed ♂, 2 months old (reckoned from last ecdysis).
Fig. 2. *A. hebraeum* Koch. Living unfed ♀, 2 months old.
Fig. 3. *A. gemma* Dönitz, scutum of living unfed ♀, about 2 weeks old.
Fig. 4. *A. hebraeum* Koch, scutum of living ♂ after sojourn of 77 days on the host.
Fig. 5. *A. gemma* Dönitz, scutum of living unfed ♂, about 2 weeks old.

PLATE II.

Fig. 1. *Dermacentor venustus* Banks, living ♂.
Fig. 2. *D. venustus* Banks, living ♀.
Fig. 3. *D. variabilis* (Say), living ♂.
Fig. 4. *D. reticulatus niveus* Neumann, living ♂.
Fig. 5. *D. reticulatus niveus* Neumann, living ♀.



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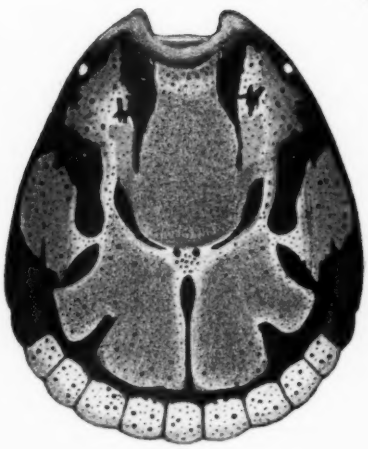

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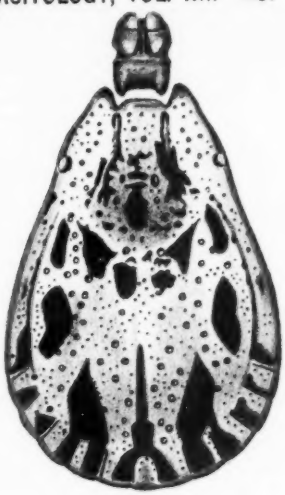

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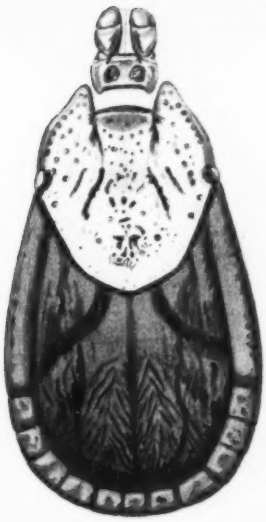

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REGENERATION OF THE MOUTHPARTS AND LEGS IN TICKS.

ARGAS PERSICUS, AMBLYOMMA HEBRAEUM AND *HYALOMMA AEGYPTIUM.*

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With 6 Text-figures.)

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INTRODUCTION.

The only authors who have concerned themselves with regeneration in Ixodoidea are Hindle and Cunliffe[1], who, at my suggestion, carried out experiments wherein they mutilated the legs of immature *Argas persicus* and observed the effects of various operations upon the development of the limbs in succeeding stages. I had laid it down as a part of the programme that these authors should also determine if the mouthparts of immature ticks are capable of being regenerated, but they confined their attention to the regeneration of the legs, it being more difficult to deal with the mouthparts especially in small larvae. Ticks are liable to be mutilated in nature by being forcibly removed from the host, whereby their mouthparts are subjected at times to

[1] Hindle, E. and Cunliffe, N. (i. 1914). Regeneration in *Argas persicus*. *Parasitology*, VI. 353–371, 4 text-figures.

considerable injury. These may remain anchored in the host's skin, when the tick's body is torn away, and every grade of injury to the mouthparts is observable in ticks that are collected in the field.

METHODS.

In all cases the ticks under experiment were operated upon by me as soon as practicable, *i.e.* an hour or two, after they had fed to repletion and had dropped off the host. A requisite number having been selected for operation, individual ticks were transferred successively to a holder that kept them in a convenient position during the brief operation which took place upon the stage of a Zeiss binocular microscope.

The holder consisted of a glass photographic plate of small size upon which a band of plasticine adhered. The tick was placed on the plasticine with its mouthparts or legs that were to be amputated projecting out over the edge of the plasticine, and a second band of plasticine, covered with a piece of fine linen, was laid upon the first and pressed down sufficiently to fix the tick in position. The tick was therefore held as it were in a vice, a soft one, the gentle grip of which could be regulated during the operation and easily readjusted if the tick shifted. The piece of linen laid on the plasticine prevented its sticking to the operator's fingers during the manipulations.

A polished scalpel was held in the left hand with the blade beneath the tick's capitulum (or legs) so that the steel could be cut down upon as on a plate by means of a fine needle held in the right hand and ground to an oblique chisel edge. Great care was taken to avoid bruising the parts or pulling them about and to make a clean cut expeditiously.

The amputated parts adhered as a rule to the scalpel through the little coelomic fluid that escaped from the tick's wound when the parts were cut, rarely did the amputated parts spring away and get lost. They were collected after each operation and mounted in balsam as follows:

A number of slides were prepared by scratching two rows of small circles (ca. 6 mm.) upon them with a writing diamond, a circle punched in a piece of card and held firmly against the slide serving as a guide to the diamond point. The slide was now reversed so that the diamond circles were on the under surface and afterwards clearly visible through the balsam mount on the upper surface, on which, at the margin of the slide and close to each circle, a number was scratched, the number corresponding to that of the tick that was to be operated upon. The slide was cleaned, and a minute drop of water was placed over the centre of a diamond circle and brought into focus upon the stage of a second dissecting microscope. The amputated parts of the tick were transferred on the point of the operating needle to the drop of water and this was allowed to dry. When the series of circles was occupied by amputated parts and these had dried, a small drop of xylol was placed upon them and this was followed by a drop of balsam. Ordinary cover-glasses cut in four yielded squares of a size that sufficed to cover the circles.

These permanent preparations, ten or more per slide, served as accurate checks to graphic notes that were made of each operation. The skins of the ticks as they moulted after operation, or the ticks themselves, were mounted serially in a similar manner, air being excluded from the mounts by taking the usual precautions. The diamond circles greatly facilitate the finding of minute objects mounted within their contours[1].

The ticks, after operation, were confined separately in numbered tubes standing in racks within a thermostat at 30° C., whence they were taken daily for inspection; the cast skins were periodically removed and mounted in the manner previously described.

The *abbreviations used in the tables* that follow are

R and L denoting Right and Left in respect to the parts that were amputated or left untouched.

— (a dash) denotes that no operation took place on the parts indicated.

The degree of amputation undergone by the several parts is denoted in different ways:

(*a*) by *fractions* of the length of the part (hypostome, palps) from apex to base, *i.e.* 1/2, 1/3, etc.

(*b*) by stating the *number of articles* that were removed wholly or partly, reckoning the basal article as 1 (palps have 4 articles 1–4) or by recording the number of distal articles removed (legs have 6 articles including the coxa).

(*c*) Operations where digits were removed without appreciable injury to the *shaft* of the chelicerae, are indicated by *cut d*; where the shaft was cut across, the amount thereof removed is given in lengths of digit taken as a measure, thus *cut* 4 *l* denotes that the portion of shaft removed with the digit was four times the length of the digit.

[1] I have devised another simple method of mounting large numbers of small objects in series. The method consists in mounting each small object in a numbered cell in balsam. To do this, cardboard of suitable thickness is cut in oblongs that are shorter (to leave room for a label) and nearly as wide as a slide. Two rows of circular holes (ca. 6 mm.) totalling 10–12 in number, are neatly cut in the card with a sharp hand-punch and serial numbers are written in Indian ink on the card beside the holes. Before use, the holed cards are immersed in xylol for 24 hours and afterwards in balsam for 24 hours; they are then transferred to a slide, the objects are placed in the holes and these are filled with balsam. A coverglass, of a size to match the card, having been dipped in xylol, is at once placed in position, the use of xylol helping to exclude air bubbles. The method is very convenient, economical, and readily lends itself to modifications.

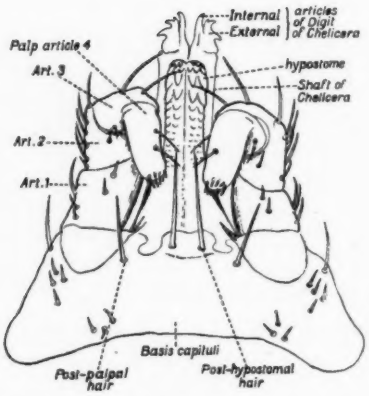

Fig. 1. *Argas persicus* ♂. Capitulum in ventral aspect, giving nomenclature of parts enumerated in the text (Nuttall, 1908).

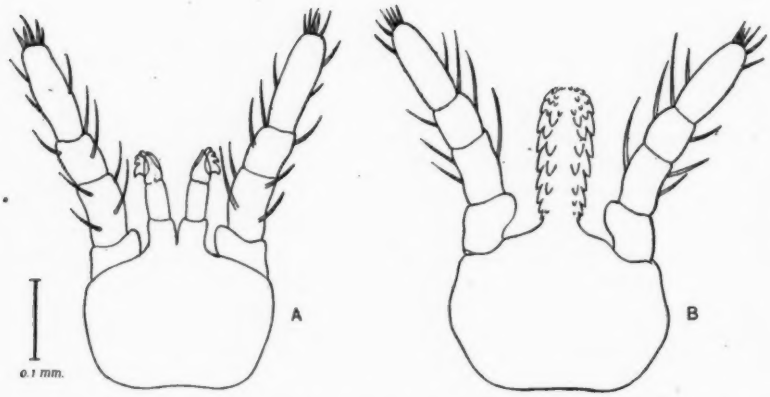

Fig. 2. *Argas persicus* larva. (A) Capitulum in dorsal and (B) in ventral aspect (Orig.).

I. REGENERATION AFTER AMPUTATION OF THE MOUTHPARTS.

(Experimental Records.)

1. Argas persicus.

(*a*) *Operations on the larva and their effect on the first-stage nymph.*

Twenty-two larvae were operated upon after feeding on a fowl. Six (Ticks 3, 6, 13, 15, 17, 19) failed to undergo metamorphosis or died soon after operation. Twelve larvae were operated upon on 22. ii. 1915 and ten on the next day. The following record relates to the sixteen specimens that survived and moulted to first-stage nymphs.

	Nature of operation on larva			Resultant effect on first-stage nymph		
Argas No.	Palp articles removed	Hypostome length removed	Chelicerae	Palps showed stumps at article	Hypostome	Chelicerae d = digit
1	R 1–4	1/3	R —	R base	perfect	L d perfect
	L 4		L cut*	L 1		
2	R —	1/4	R cut	R 2†	perfect	R and L d deformed
	L 2–4		L cut	L 1		
4	R 2–4	at base	R —	R 3	regen. but	L d deformed
	L 1–4		L cut 1 *l*	L 3	deep emarg.	
5	R 2–4	2/3	R —	R 1 (fig. 4 B)	perfect	—
	L 2–4		L —	L 1		
7	R —	at base	R —	R deformed†	deformed	—
	L 2–4		L —	L 1	(see fig. 4 G)	
8	R 2–4	3/5	R —	R 1	perfect	—
	L —		L —	L 1		
9	R 2–4	1/4	R cut	R and L 1	perfect	R d deformed (fig. 4 K)
	L 2–4		L cut	(fig. 4 D)		L d ?
10	R 2–4	at base	R cut ½ *l*	R and L 2	regen. but	R d distinctly, and
	L 2–4		L cut 1 *l*	(fig. 4 C)	some asym-	L d slightly deformed
					metry	(fig. 4 J)
11	R 3–4	2/5	R —	R 2	perfect	—
	L 3–4		L —	L 2		
12	R 3–4	1/4	R cut	R and L 3	perfect	—
	L 3–4		L cut	(fig. 4 E)		
14	R —	1/4	R cut 4 *l*	—	regen. slight	R d not regen. (see fig. 4 M)
	L —		L —		asymmetry	
16	R —	2/5	R cut 1 *l*	R deformed but	regen. slight	R d deformed
	L —		L cut 1 *l*	of normal length†	emarg.	L d deformed
18	R 2–4	at base	R cut 1 *l*	R 1	perfect	R d deformed
	L 2–4		L cut 1 *l*	L 1		R d distinctly deformed
20	R —	near base	R cut d	—	perfect	R d deformed
	L —		L cut 1 *l*			L d deformed, useless
21	R —	2/5	R cut 1 *l*	—	regen. slight	R d slightly deformed
	L —		L cut 1 *l*		asymmetry	L d „ „
					(fig. 3 A)	
22	R 2–4	2/5	R cut	R 1	do.	R d useless (fig. 4 L)
	L crushed?		L cut	L 2†		L d slightly deformed

* Owing to loss of some amputated parts of chelicerae it is impossible in all cases to specify how much was removed.

† The tick must have been unwittingly injured at operation.

(b) Operations on first-stage nymphs and their after effects.

Twenty-one first-stage nymphs were operated upon on 15–16. iii. 1915, after feeding on a fowl.

	Nature of operation on first-stage nymph			Resultant effect on after stages				
Argas No.	Palp articles removed	Hypostome cut at	Chelicerae digits	Moulted on day and month (1915)	Palps showed stumps at article	Hypostome	Chelicerae digits	Subsequently fed, and developed perfectly, moulted on
28	R — L 2–4	base	R — L —	4. iv.	R L 4	perfect	—	7. v. ♂
29	R 2–4 L —	base	R hurt? L —	29. iii.	R stumpy	perfect	perfect	28. v. ♀
30	R hurt? L —	base	R cut d L —	4. iv.	R 2	regen., emarg.	R perfect	1. vi. ♀
31	R 3–4 L —	base	R — L —	1. iv.	R stumpy	perfect	—	10. v. ♂
32	R — L 2–4	base	R — L —	29. iii.	L short	perfect	—	8. v. 3rd-st. nymph
33	R 3–4 L —	base	R hurt? L —	26. iii.	R 3	perfect	—	8. v. ♀
34	R — L —	base	R cut 1 *l* L cut	30. iii.	—	perfect	R slightly def. L perfect?	8. v. ♀
35	R — L —	base	R cut d L —	29. iii.	—	regen., emarg.	R. v. slightly deformed	26. v. 3rd-st. nymph, refused to feed
36	R — L —	base	R cut 1 *l* L cut d	1. iv.	—	perfect	R slightly def. L deformed, small	11. v. ♀
37	R — L —	base	R cut d L —	30. iii.	—	normal?	R (lost)	4. vi. ♀
38	R — L —	base	R cut 1 *l* L cut 1 *l*	30. iii.	—	slightly emarg.	R and L (lost)	28. v. ♂
39	R — L —	base	R — L —	30. iii.	—	do.		28. v. ♂
40	R — L —	base	R cut 2 *l* L cut 1 *l*	2. iv.	—	perfect	R d deformed L d normal	2. vi. ♂
41	R — L —	base	R cut d L —	30. iii.	—	perfect	R d deformed	10. v. ♀
42	R — L —	base	R cut 1 *l* L cut d	2. iv.	—	perfect	R and L slightly deformed	11. v. ♀
43	R — L 2–4	base	R cut d L —	29. iii.	L regen. but short	perfect	R d perfect	20. vi. ♂
44	R — L 4	base	R — L cut d	30. iii.	do.	perfect	L d deformed	10. v. ♂
53	R 2–4 L —	base	R cut d L —	3. iv.	R few sensory hairs at tip	perfect	R d deformed	22. vi. ♀
55	R — L 2–4	base	R cut 1 *l* L cut 1 *l*	1. iv.	L imperfect	perfect	R and L d slightly deformed	1. vi. ♂
59	R 3–4 L 1–4	—	R cut d L —	1. iv.	R 4 L 2	regen., emarg.	R and L slightly small	31. v. ♂
62	R 2–4 L 2–4	base	R cut d L cut d	2. iv.	R and L small?	perfect	R and L deformed	12. v. ♀

Amputations through the basis capituli.

Ten first-stage nymphs had the basis capituli cut through transversely on 15. iii. 1915. Only four survived this severe operation which was accompanied by a considerable loss of coelomic fluid.

Argas No.	Cut across basis capituli	After effects on 2nd-stage nymph
50	Cut diagonally near base of palps, including a long piece of shaft and digits of chelicerae.	19. iv. 15. Found partly moulted, feeble. Exuviae were carefully removed and found palps absent; hypostome represented by a smooth stump; chelicerae digitless, the tip of one sheath toothed, the other rounded.
51	Cut similarly to No. 50 but more basally.	19. iv. Found partly moulted, feeble, without capitulum, hypostome or palps, whilst two flattened digitless cheliceral sheaths protruded like fingers from a glove.
54	Cut similarly to the last.	5. iv. Moulted and died on 30. iv. Palp absent on the right side and reduced to a thin finger-like process on the left side; hypostome absent; chelicera of left side with its digit very small, the digits on both sides deformed (see Fig. 3 B and B′).
60	Cut across anteriorly, a good portion of cheliceral shaft being included.	1. iv. Found moulting, thin and weak; it was helped to free itself. The tick died on 28. iv. Whilst it possessed neither hypostome nor palps, the chelicerae appeared normal.

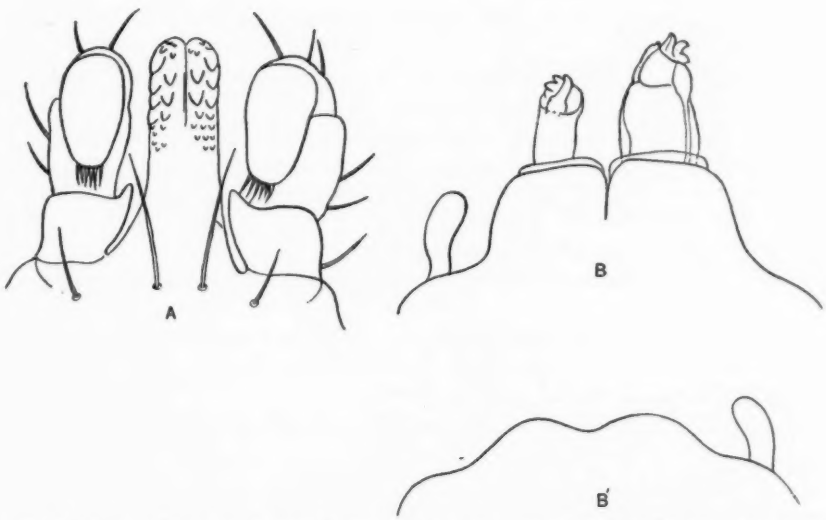

Fig. 3. *Argas persicus* nymphs. (A) Capitulum of 1st-stage nymph which had its hypostome and digits removed when in the larval stage (*Argas* 21); ventral aspect. (B) Capitulum of a 2nd-stage nymph which had its basis capituli cut across when in the first nymphal stage (*Argas* 54); dorsal aspect. (B′) Ventral aspect of the same, showing absence of hypostome, one palp reduced to a finger-like process and the other absent, digits deformed.

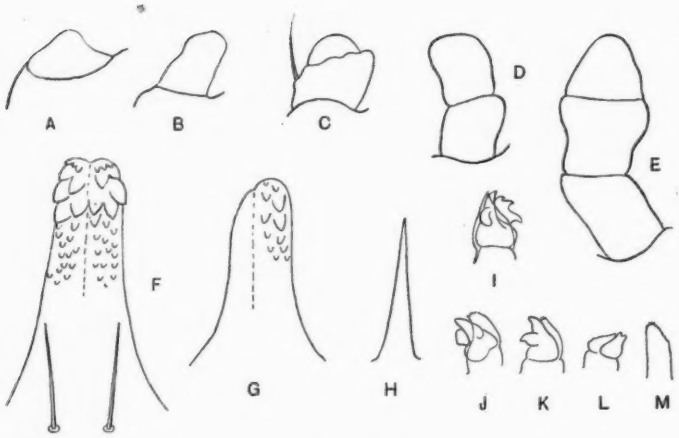

Fig. 4. *Argas persicus* nymphs. Illustrating effects of mutilations inflicted in preceding stages:

(A) *Argas* 22, right palp in ventral aspect. First-stage nymph operated upon as a larva. Article 1 partly regenerated.

(B) *Argas* 5, ditto. The left palp closely resembled the one figured at (A). Partial regeneration of article 1.

(C) *Argas* 10, ditto. The left palp presented a similar appearance in this specimen. Regeneration of article 1 and a stump of article 2.

(D) *Argas* 9, ditto. The left palp was similarly formed. Articles 1 and 2 regenerated.

(E) *Argas* 12. Left palp in ventral aspect. First-stage nymph that had been operated upon as a larva. Articles 1 and 2 regenerated but malformed; article 3, partly regenerated, forms the stump.

(F) Hypostome of *normal* 1st stage nymph.

(G) Deformed hypostome of *Argas* 7, a 1st-stage nymph that had been operated upon as a larva.

(H) Much deformed hypostome of *Argas* 67, a 3rd-stage nymph that had been operated upon as a 2nd-stage nymph. The structure forms a toothless spine.

(I) Digit of *normal* 1st-stage nymph in dorsal aspect.

(J–K–L) Deformed digits of 1st-stage nymphs, in dorsal aspect, as they appeared in *Argas* 10, 9, 22 that had been operated upon as larvae.

(M) Digitless end of right chelicera in *Argas* 14, a 1st-stage nymph that had been operated upon as a larva.

(*c*) *Operations on second-stage nymphs and their after effects.*

Fifteen second-stage nymphs were operated upon on 29. iv. 1915 after feeding on a fowl. The following record relates to ten of these that survived and moulted to adults or third-stage nymphs which were subsequently raised to adults.

	Nature of operation on second-stage nymph				
Argas No.	Palp articles removed	Hypostome cut off at	Chelicerae	Moulted on	Resultant effect on after stages
63	R 2–4 L —	base	R and L cut d	18. v. 15	perfect ♂.
65	R and L 2–4	base	R cut d L —	18. v. 15	perfect ♂.
67	R at base L at base	base	R cut d L cut	25. v. 15	A 3rd-stage nymph with palps regenerated, hypostome a toothless spine (fig. 4 H), digits normal. Refused to feed and finally died.
68	R 2–4 L 1–4	base	R and L —	15. v. 15	♀, normal but for asymmetric emarginate hypostome. Fed 28. v.
70	R 2–4 L 1–4	base	R and L cut	17. v. 15	3rd-stage nymph normal but for somewhat asymmetric emarginate hypostome and rather small teeth on the external article of digits. Fed on 28. v.
74	R 2–4 L 2–4	base	R and L cut d	14. v. 15	3rd-stage nymph, perfect; fed 3. vi. and moulted 17. vi. as a normal ♀.
75	R 2–4 L 3–4	base	R cut 1 *l* L —	22. v. 15	3rd-stage nymph, perfect.
76	R 2–4. L 2–4	1/2	R cut L —	18. v. 15	3rd-stage nymph, perfect.

Amputations through the basis capituli.

Three second-stage nymphs had the basis capituli cut across on 29. iv. 15, and two survived the operation:

Argas No.	Cut across basis capituli		After effects on third-stage nymph
72	Cut diagonally from behind one palp to base of other and through digits and pieces of cheliceral shafts.	Tick moulted on 19. v.	Fed on 28. v. The tick was perfectly formed, the external article of the digits was somewhat small.
73	Cut midway across its length and through digits and cheliceral shafts to thrice the length of digit.	Tick moulted on 25. v.	Refused to feed 28. v. Its digits were deformed, the hypostome was absent, the palps merely represented by small protrusions. It survived in this condition until 13. v. 1919 (*i.e. four years* unfed) when it was killed and preserved.

2. Amblyomma hebraeum.

(*a*) *Operations on the larva and their effects on the nymph.*

Thirteen larvae were operated upon on 27. vii. 1915, after they had fed to repletion upon a hedgehog and dropped off the host.

	Nature of operation on larva			
Amblyomma larva No.	Palps	Hypostome cut away	Chelicerae digits	Regeneration observed in nymph
1	R and L —	at base	L cut 4 *l*	perfect
2	R and L —	at base	R and L cut 3 *l*	perfect
3	R and L —	at base	R and L cut 3 *l*	perfect
4	R and L —	1/2	R cut 2 *l* L cut ½ *l*	perfect
5	R and L —	at base	R cut 3 *l*	R chelicera shaft 1/4 shorter than normal, otherwise perfect
6	R cut at base L cut near base	1/2	R cut ½ *l* L cut 2 *l*	perfect
7	R cut at base L —	3/4	R and L cut 2 *l*	perfect
8	R and L —	3/4	R cut 1 *l* L cut 2 *l*	perfect
9	R — L cut ½	at base	R cut 2 *l* L cut 2 *l*	perfect
10	R — L at base	at base	R cut 2 *l* L —	perfect

(*b*) *Operations on the nymph and their effects on the adult.*

Ten nymphs were operated upon on 3. viii. 1915, after feeding to repletion on a hedgehog.

	Nature of operation on nymph			
Amblyomma nymph No.	Palp articles cut away	Hypostome cut across	Chelicerae length of shaft cut in terms of digit length	Regeneration observed in adult ♂ or ♀
1	R and L —	at base	R and L cut 4 *l*	♂ perfect
2	R and L —	at base	R cut 2 *l* L cut 3 *l*	♂ perfect
3	R and L cut 2–4	under base	R and L cut 2 *l*	♀ perfect
4	R — L cut 2–4	at base	R and L cut 1 *l*	♀ perfect
5	R and L 2–4	at base	R cut 1 *l* L cut 3 *l*	♀ perfect
6	R cut 3–4 L cut 2–4	at base	R — L cut 2 *l*	♀ perfect
7	R cut 2–4 L cut 1–4	at base	R and L cut 2 *l*	♂ perfect

3. Hyalomma aegyptium.

Operations on the nymph and their effects on the adult.

Seventeen nymphs were operated upon on 1. vi. 1915, after feeding and dropping from a hedgehog. They all moulted between 17 and 22. vi., except No. 17 which moulted on 24. vi. 15.

	Nature of operation on nymph			
Hyalomma No.	Palp articles removed	Hypostome cut	Chelicerae	Regeneration observed in adult ♂ or ♀
1	R and L —	nr base	R and L cut 2 *l*	♀ right internal article of one digit slightly deformed, otherwise perfect
2	R and L —	nr base	R cut 1 *l*	♀ perfect
3	R and L —	nr base	R cut 2 *l* L cut 1 *l*	♀ perfect but for deformity of external article of right digit
4	R and L —	nr base	R and L cut 2 *l*	♀ perfect
5	R and L —	at base	R cut 3 *l*	♀ perfect
6	R cut 2–4 L —	nr base	R and L cut 2 *l*	♂ perfect
7	R cut 1–4 L —	nr base	R cut	♀ perfect but for deformed R internal article of digit
8	R and L cut 2–4	at base	R and L cut 4 *l*	♀ R and L digits badly deformed, rest normal
9	R and L —	nr base	R cut	♀ perfect
10	R and L —	nr base	R and L cut 1 *l*	♀ perfect

II. REGENERATION AFTER AMPUTATION OF THE LEGS.

(Experimental Records.)

The experiments of Hindle and Cunliffe (cited on p. 7) on the mutilation of the legs in *Argas persicus* may be summarized as follows:

When the legs of a freshly gorged larva are amputated, they are not regenerated or are imperfect in the first-stage nymph. If the larva is operated upon whilst on the host, that is 2–3 days *before* it would drop off gorged if left unmolested, the legs may at times be regenerated after the tick abandons the host. This difference in the behaviour of the larva under the two conditions specified is attributed to nymphal development proceeding within the larva whilst it is upon the host and developing nymphal tissues being injured when gorged larvae are operated upon. First-stage nymphs that were mutilated in the larval stage, when raised, without further operative interference to second-stage nymphs, were found to have regenerated the amputated limbs although these were usually of subnormal size whilst perfectly formed; after a further moult, the ticks became normal. Experiments were also made with nymphs. It was found that leg regeneration occurred in all immature stages where amputation took place sufficiently long before moulting, but the legs were usually of subnormal size.

In my experiments the legs were amputated in immature stages of *Amblyomma hebraeum*, *Hyalomma aegyptium*, and in a few *Argas persicus* for purposes of comparison:

1. Argas persicus.

(*a*) *Operations on the larva and their effect on the first-stage nymph.*

Legs were amputated in three larvae (Nos. 23, 24, 26) on 23. ii. 15. In No. 23 legs I–III on the right side had 4, 3½, and 4 distal articles cut off respectively; in No. 24 leg III had 2½ articles removed; in No. 26 leg I had 4 articles removed.

In none of these larvae were the limbs regenerated in the first-stage nymph, all of the limbs ended in stumps corresponding to the seat of amputation in the larva. No. 26 moulted as a first-stage nymph on 17. iii., it fed on 25. iii., and on 19. iv. it was found to have moulted with all its legs normally formed.

(*b*) *Operations on the first-stage nymph and their effect on the second-stage nymph.*

The legs of the right side were amputated in three first-stage nymphs (Nos. 45–47) on 25. iii. 15. In No. 45 legs I and IV had 5 articles removed; in No. 46 legs I and II had 5 and 3 articles removed respectively; in No. 47 legs I and III had 4 articles removed. The three ticks moulted to second-stage nymphs on 30. iii.–2. iv. and fed normally on 28–30. iv.

In the second nymphal stage, No. 45 had legs I and IV of subnormal size, and No. 46 had legs I and II smaller than the corresponding legs on the side that had not been mutilated. Through an oversight No. 47 was allowed to moult and the moulted legs were lost so that its condition escaped observation.

After further feeding, Nos. 45 and 47 moulted to third-stage nymphs whilst No. 46 emerged as a normal ♂, skipping the third nymphal stage which is more commonly omitted in normal ticks of this species. No. 47 gave rise to a ♀. In all of these stages the ticks appeared perfectly formed, no difference in size between corresponding legs being observable.

2. Amblyomma hebraeum.

(*a*) *Operations on the larva and their effect on the nymph.*

Legs were amputated in three larvae (Nos. 11–13) on 27. vii. 15 as follows: In No. 11 the greater part of legs II and III was removed; in No. 12 leg I had 5 articles removed; in No. 13 leg III had 5 articles removed.

Examined after they had moulted, the nymphs were found to have regenerated their legs perfectly, the previously mutilated limbs not being smaller than normal.

(*b*) *Operations on the nymph and their effect on the adult.*

Legs were amputated in three nymphs (Nos. 8–10) on 3. viii. 15 as follows: In No. 8 legs III and IV had 3 and 4 articles removed respectively; in No. 9 legs II and III lost 3 and 4 articles respectively; in No. 10 leg I had 4 articles cut off. These ticks in due course moulted and gave rise to three females.

All of the previously amputated limbs were regenerated in the adults, only in No. 9 were legs II and III (those mutilated) slightly smaller than normal.

3. Hyalomma aegyptium.

Operations on the nymph and their effect on the adult.

The legs were amputated in seven nymphs (Nos. 11–17) as follows:

Hyalomma No.	Number of articles amputated from legs	Regeneration in adult
11	2 from leg III	perfect
12	2 from leg IV	„
13	4 from legs II and III	„ (untouched opposite leg III small)
14	4 from leg I	„
15	3 from leg II	regenerated, distal articles very slightly shorter than normal
16	4 from legs I and II	regenerated, slightly shorter than normal
17	3–4 from legs I, II, III, IV	regenerated, legs II, III, IV somewhat smaller than normal

SURVEY OF THE RESULTS OBTAINED.

All the immature ticks in these experiments were operated upon within 1–2 hours of their becoming fully gorged and abandoning the host. Extensive mutilations may cause death through excessive loss of coelomic fluid. Moderate mutilations are well borne by ticks, 78 out of 108 survived the operations herein described; the heaviest loss followed operations affecting the basis capituli. One third-stage nymph (*Argas* 73), in which the basis had been cut across midway in the second nymphal stage, survived for four years in the laboratory although it was unable to feed because of its mutilated mouthparts[1]. Metamorphosis may be retarded or not according to the severity of the mutilation that has been inflicted.

REGENERATION OF MOUTHPARTS.

Argas persicus.

Operations on *larvae* wherein the mouthparts are mutilated shortly after the larva has abandoned the host in a fully gorged condition, cause the various structures to be differently affected in the first-stage nymph: the *palps* are not regenerated but appear as stumps which but for their closed and rounded ends correspond mostly in structure to the parts that were left intact in the larva (Fig. 4 A–E); occasionally an additional article or two is

[1] See p. 24.

regenerated. The *hypostome*, when amputated at any point short of its base, is perfectly regenerated, but if cut at its base the regenerated hypostome may be slightly deformed (Fig. 3 A). It should be noted that slight asymmetry and irregularities of dentition occur in all stages normally. The *digits of the chelicerae* are usually deformed to a varying degree (Fig. 4 D, K, L), the greatest deformity or even their non-regeneration may be caused by cutting off any considerable length of the shaft (Tick 14, Fig. 4 M); slightly deformed digits may function well enough for purposes of feeding.

First-stage nymphs, when mutilated as described in the case of the larvae, yield second-stage nymphs whose structures are affected as follows: the *palps* may appear like the stumps above described, they may be partly regenerated (*Argas* 28, 33, 53, 59), they may be completely regenerated whilst appearing short but about as broad as normal (*Argas* 32, 43), or they may appear normal. The *hypostome* in most cases is perfectly regenerated, but at times it is emarginated distally, an appearance occasionally met in ticks that are presumably normal. The *digits of the chelicerae* may be regenerated perfectly, or they may be undersized or deformed even when a length of shaft only equal to one or two digit-lengths is removed at operation. Slightly deformed digits do not prevent the mutilated ticks from feeding normally.

The whole series of second-stage nymphs, when left unmolested after the first operation, and adequately fed, gave rise to normal adults (19 specimens) or third-stage nymphs (two specimens) at the next moult.

When the *basis capituli* was cut across in first-stage nymphs only four out of ten of the ticks survived, the operation being accompanied by a great loss of coelomic fluid. None of the survivors moulted normally. In one case the basis capituli was not regenerated (*Argas* 51), in only one case was there an abortive attempt to regenerate a palp (*Argas* 54, Fig. 3 B and B′); the hypostome was not regenerated, at most, in one case it was represented by a smooth stump (*Argas* 50); the sheaths of the chelicerae were regenerated, they terminated either with apparently normal digits (*Argas* 60), small or deformed digits (*Argas* 54, Fig. 3 B), or they were devoid of digits (*Argas* 50, 51), these differences depending no doubt upon the degree of mutilation to which the parts had been subjected according as they were more or less protruded when amputated.

Second-stage nymphs, when mutilated, yielded adults (three specimens) or third-stage nymphs (five specimens) at the succeeding moult. In these the *palps* were all perfectly regenerated, in one tick the palp had been amputated basally. The *hypostome*, cut across basally, was perfectly regenerated in five cases, it appeared emarginated and asymmetrical in two, and in only one case did it subsequently develop to a toothless spine (*Argas* 67, Fig. 4 H). The *digits of the chelicerae* were regenerated perfectly in all but one case (*Argas* 70) wherein the external article of the digits appeared somewhat small. Only one of these ticks refused to feed (*Argas* 67 above referred to) and those that emerged as third-stage nymphs were subsequently raised to perfect adults.

When the *basis capituli* was cut across in second-stage nymphs, as was done successfully in two instances, almost perfect regeneration followed in one case (*Argas* 72), whilst in the other, where the basis was cut across half-way, the palps only reappeared as short stumps, the hypostome was absent and the digits deformed.

The results obtained with *Argas*, in respect to the power possessed by various immature stages of regenerating their mouthparts, are best elucidated by the following summary relating to the milder operations described in the foregoing pages:

OPERATIONS ON LARVAE		RESULT IN NEXT STAGE
Palps amputated	21	2 regenerated partly 19 not regenerated
Hypostomes amputated	16	16 regenerated 9 perfect 5 slightly deformed 2 deformed
Digits amputated	18	17 regenerated 5 slightly deformed 8 deformed 4 much deformed 1 *not* regenerated
OPERATIONS ON FIRST-STAGE NYMPHS		
Palps amputated	13	11 regenerated 2 perfect 5 short or imperfect 4 partially regenerated 2 *not* regenerated
Hypostomes amputated	20	20 regenerated 15 perfect 5 slightly deformed
Digits amputated	17	17 regenerated 5 perfect 6 slightly deformed 6 deformed or small
OPERATIONS ON SECOND-STAGE NYMPHS		
Palps amputated	15	15 regenerated 15 perfect
Hypostomes amputated	8	8 regenerated 5 perfect 2 slightly deformed 1 badly deformed
Digits amputated	11	11 regenerated 9 perfect 2 slightly deformed

It should be noted, at the outset, that operations on *larvae* which have dropped off in a replete state from the host yield, on the whole, unfavourable

results in respect to regeneration for the reasons specified on pp. 17 and 23. It is noticeable that after operations on larvae the hypostome is regenerated better than the digits, whilst mostly no attempt at regeneration takes place in the palps.

When the *first-stage nymph* has its mouthparts amputated, the hypostome is best regenerated, then follow the digits, whilst the palps are least well regenerated.

This order changes, however, when operations are made in the *second-stage nymphs*, for the succeeding stage (adult or third-stage nymph) shows regeneration to have taken place best in the palps and digits and least well in the hypostome. The cause of this phenomenon requires elucidation.

The foregoing tabular summary shows, moreover, that as the tick develops toward maturity, the power to regenerate the palps and digits increases, there being no distinct difference in respect to the hypostome, although the proportion of perfect to slightly imperfect hypostomes in second-stage nymphs after operation is highest, *i.e.* 15 : 20.

Amblyomma hebraeum.

Operations on *larvae*, consisting of basal amputations of palps and hypostome, were followed in the nymph by perfect regeneration in all cases (ten operations); amputations of digits and twice their length of cheliceral shaft had no effect, but in one case, where a longer piece of shaft was removed (*Amblyomma* 5), the shaft appeared shorter than normal in the nymph.

Operations on *nymphs*, consisting of basal amputations of palps and hypostome or of digits with four times their length of shaft, were followed by complete regeneration of all parts in the adult.

Hyalomma aegyptium.

Operations on *nymphs* (ten specimens) wherein the palps and hypostome were amputated at or near the base, were followed by perfect regeneration of these parts in the adult. Amputations of the digits and 2–3 times their length of cheliceral shaft, only resulted in deformity of the digit in one case (*Hyalomma* 3), whereas, when more of the shaft was removed (*Hyalomma* 8), the digits appeared badly deformed in the adult.

The results obtained after amputations of mouthparts in the Ixodid ticks are therefore in strong contrast to those in *Argas*. Regeneration takes place equally well after operations on larvae and nymphs. Perfect regeneration followed on almost all operations, *i.e.* 27 hypostome amputations, 23 palp amputations and 40 amputations of digits; slight deformity of digits followed three operations, great deformity in two, and in but one case was the cheliceral shaft shortened through mutilation.

REGENERATION OF LEGS.

Argas persicus. It was found that when freshly gorged larvae had their legs amputated, the corresponding limbs were not regenerated in the first nymphal stage, but when these nymphs were fed and allowed to undergo a further moult without operative interference, they regenerated these mutilated limbs perfectly. When first-stage nymphs, under like conditions, were similarly mutilated, they gave rise to second-stage nymphs with well-formed legs of subnormal size. These results are in conformity with those of Hindle and Cunliffe (cited on p. 7) who explain the difference in the results obtained with larvae and nymphs by an interesting observation on larvae subjected to operations made at an earlier stage whilst still feeding on the host. When replete larvae abandon the host, they are well advanced in their nymphal development, and, consequently, if they are operated upon at this stage, they do not regenerate their legs, the first-stage nymphal formative tissues having been injured.

Amblyomma hebraeum. My experiments on this species show that when the legs are mutilated in replete larvae or nymphs immediately after the ticks have abandoned the host, the limbs are regenerated in the succeeding stage; in one adult that developed from a nymph which had been mutilated, the regenerated limbs were slightly smaller than normal, in the remaining six ticks the regenerated limbs were of normal size.

Hyalomma aegyptium. Similar experiments of mine upon replete nymphs of this species show that the regenerated limbs in the adult may be either perfect or of subnormal size.

Therefore 21 leg amputations in Ixodid ticks were followed in all cases by regeneration, 13 legs being normal and 8 slightly reduced in size.

SUMMARY AND CONCLUSIONS.

Amputation experiments upon immature stages of *Argas persicus* (Oken 1818), *Amblyomma hebraeum* Koch 1844, and *Hyalomma aegyptium* (Linnaeus 1746) show that the mouthparts and legs of these ticks may be more or less regenerated when mutilated shortly after the ticks have abandoned the host in a fully engorged condition.

Regeneration of Mouthparts.

In immature *Argas persicus* the mouthparts are regenerated more or less perfectly according to the structure affected and the stage of development of the tick. Freshly gorged larvae regenerate their mouthparts badly compared to first and second-stage nymphs because during their period of parasitism upon the host (5–6 days usually) the larvae have advanced well along the road to becoming nymphs, therefore the operation largely affects the formative tissues of the developing nymph. The nymphs feed rapidly, usually in

15–20 minutes, and proceed with their metamorphosis after abandoning the host, consequently, when they are mutilated soon after feeding, they regenerate their mouthparts (and legs) better than do the larvae. After operations on larvae and first-stage nymphs regeneration takes place best in the hypostome, next in the chelicerae and least well in the palps. This order was reversed after similar operations on second-stage nymphs. The power to regenerate palps and chelicerae grows as the tick approaches maturity, but it remains fairly constant for the hypostome. Amputations made through the basis capituli usually render the tick incapable of feeding after it has moulted to the next stage, the mouthparts not having been adequately regenerated. In one case (*Argas* 72), however, the amputation of the anterior part of the capitulum with its appendages in a second-stage nymph was followed by almost perfect regeneration of the part in the succeeding third-stage nymph. Where regeneration did not take place adequately after severer operations on the basis capituli, as exemplified in another case (*Argas* 73), the mutilated tick lived on without its capitulum for four years without a meal. This experiment explains the origin of the anomalous "headless female" of *Ixodes ricinus*, referred to and figured by Wheler[1], which lived four years and was lost. Lesser mutilations, *i.e.* those affecting the appendages only, are frequently followed by perfect regeneration if the injury is slight. Imperfectly regenerated parts are often capable of functioning so that the tick can feed. Such a tick, if not interfered with, will acquire perfectly normal mouthparts at the next moult.

The immature stages of the Ixodid ticks *A. hebraeum* and *H. aegyptium* behave differently to *Argas persicus* when injured soon after abandoning the host, in that they possess much greater powers of regeneration. Basal or partial amputations of the hypostome and palps, or moderate mutilations of the chelicerae, are followed by perfect regeneration in most cases.

The greater power of regeneration possessed by Ixodid ticks bears directly upon their parasitic habits. The slowly feeding larval stages of Argasids (*A. persicus*, *A. vespertilionis*, for example) possess relatively more dentate hypostomes than do the adults which are rapid feeders. With the exception of *Haemaphysalis concinna* (vide Brumpt's observations recorded by me in *Parasitology*, VII. 434) all known Ixodids are slow feeders and an examination of genera like *Ixodes*, *Amblyomma* and *Hyalomma* proves that their hypostomes are most efficient organs for anchoring the tick to the host. The accompanying illustrations (Figs. 5 and 6) of the capitulums of *A. hebraeum* and *H. aegyptium* females show that they possess long, highly dentate hypostomes broadening distally and liable to break where they narrow toward the base when violence is done to the tick that is fixed thereby to the skin. The examination of such Ixodid ticks collected in the field proves how frequently their long hypostomes get broken off near the base, and consequently how vital it is that they should be readily regenerated. Whilst a tick may save

[1] Wheler, E. G. (iii. 1906). British Ticks. *Journ. of Agricult. Sci.* I. p. 401, Plate X, fig. 38.

its digits by moving and retracting them, it cannot do so with its hypostome which is a rigid structure whose chitin is continuous with that forming the basis capituli. The hypostome is the most readily regenerated element of the mouthparts because it consists almost entirely of chitin, its formative tissues lying beneath the stout chitinous base whilst it is developing in the

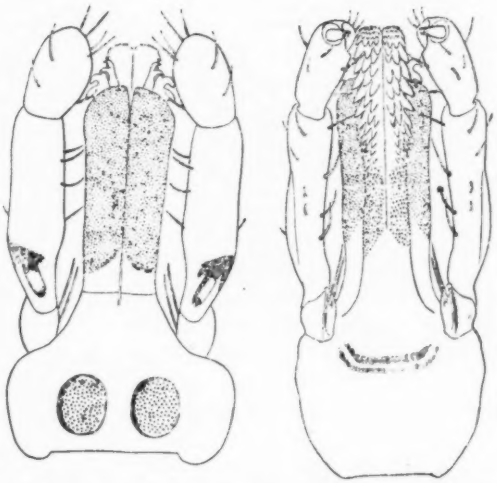

Fig. 5. *Amblyomma hebraeum* ♀. Capitulum in dorsal and ventral aspect (Original, ca ×16).

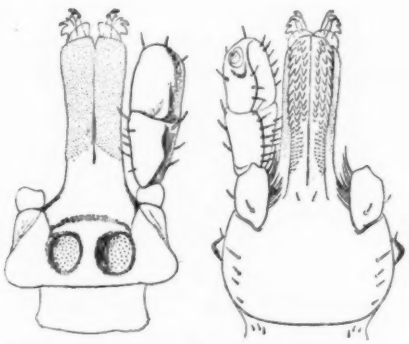

Fig. 6. *Hyalomma aegyptium* ♀. Capitulum in dorsal and ventral aspect (Nuttall, 1911 ca. ×16).

immature stages prior to a moult. In this position, the formative tissues are not liable to be hurt, whereas in the case of the palps and chelicerae as with the legs, the formative tissues are more directly accessible to injury.

The power of regenerating mutilated mouthparts possessed by immature ticks is of paramount importance in connection with their maintenance in

nature where they are frequently injured through their forcible removal from the host, the hooked hypostome and digits of the chelicerae being broken off to a varying degree because they are so firmly anchored in the host's skin. Such mutilation of the mouthparts is much more likely to occur in Ixodid than in Argasid ticks because the mouthparts of the latter are as a rule less effective anchoring organs, the structure of the mouthparts in the two groups being correlated with their feeding habits upon the host.

Regeneration of legs.

The experiments with *Argas persicus* herein recorded confirm those of Hindle and Cunliffe (*loc. cit.*). If the legs of the larva are amputated shortly after the tick has abandoned the host in a fully gorged condition, the first-stage nymph usually shows stumps corresponding in length to the portion of limb that was left intact in the larva. The authors cited found, however, that if the larva had its legs amputated whilst attached and feeding on the host, *i.e.* 2–3 days prior to its dropping off in a replete state, that the legs may at times be regenerated in which case they are usually of subnormal size. When the legs of first and second-stage nymphs are amputated soon after they have fed, the limbs are regenerated but are usually small. Immature ticks with small or stumpy legs, if not subjected to further interference, develop normal limbs after a further moult.

My experiments with *A. hebraeum* and *H. aegyptium* show that these Ixodid ticks possess greater powers of regeneration than *A. persicus* in respect to the legs, this being in harmony with the results above described in connection with the mouthparts. The number of legs amputated from the two Ixodid species in the larval and nymphal stages was 21 and all of them were regenerated; 13 limbs were of normal size and 8 slightly smaller than normal.

SCLEROSTOMES OF THE DONKEY IN ZANZIBAR AND EAST AFRICA.

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(With 5 Text-figures.)

INTRODUCTION.

During the last few years our knowledge of the Sclerostome parasites of the horse has been greatly augmented, the nematodes of the donkey have not however received attention since 1901, the date of publication of Looss' famous monograph on the Sclerostomes of the Equidae.

I have recently received for study two collections of helminths from domestic animals, each of which includes a number of nematodes from the donkey; for the first of these I am indebted to Mr W. M. Aders, Economic Biologist of the Zanzibar Government, the second collection was kindly entrusted to me by Professor G. H. F. Nuttall, F.R.S., who had received it from Mr R. Eustace Montgomery of the Veterinary Pathological Laboratory, Nairobi.

Some of the material from the latter source reached me in a poor state of preservation and it was not possible to identify all the forms present, altogether however nine species were observed, including two hitherto undescribed:

1. *Strongylus vulgaris* (Looss).
2. *Strongylus edentatus* (Looss).
3. *Strongylus asini* sp. n.
4. *Triodontophorus intermedius* Sweet.
5. *Cylicostomum auriculatum* (Looss).
6. *Cylicostomum coronatum* (Looss).
7. *Cylicostomum bicoronatum* (Looss).
8. *Cylicostomum alveatum* (Looss).
9. *Cylicostomum adersi* sp. n.

The worms numbered 1–3 occurred in both collections, 4–8 were from East Africa, whilst No. 9 was from Zanzibar only.

Since the publication of Looss' monograph a number of new species have been referred to the genera *Triodontophorus*, *Gyalocephalus* and *Cylicostomum*,

and other additions will no doubt be made, especially to the last-named genus, as more material from horses and donkeys is investigated; it is however interesting to find an undescribed species among the larger Sclerostomes belonging to the genus *Strongylus* s.s.

The new species from the donkey is a large form, in some respects intermediate between *Strongylus equinus* (Looss) and *S. edentatus* (Looss), but possessing some characteristic features peculiar to the species, it will no doubt be found not to be restricted to that host but to occur in the horse as well.

Owing to the thickness of the head and neck muscles in the larger forms of *Strongylus* I have found the anatomy of these worms somewhat difficult to make out in glycerine mounts and I have found it desirable to examine them in a medium of rather higher refractive index. For this purpose pure white creasote (into which the worms may be transferred direct from alcohol without shrinkage) has proved very suitable. I have to thank Mr H. A. Baylis, of the British Museum, for calling my attention to this very useful reagent.

Genus STRONGYLUS Mueller

(= *Sclerostoma* Rudolphi).

Strongylus asini sp. n.

SPECIFIC DIAGNOSIS. *Strongylus*: Body large, 18–42 mm. in length with a maximum thickness of 1·8–2·5 mm.

The head (0·6–1·3 mm. in breadth) is divided from the rest of the body by a slight constriction to form a neck-like region, similar to, but not nearly so well defined as that of *S. edentatus*.

The mouth is circular and of considerable size (320–470μ in diameter). The mouth collar is deep and sharply marked off from the rest of the skin.

External and internal leaf-crowns are of the type usual in *Strongylus*, especially resembling those of *S. equinus*. The lateral and submedian head-papillae are also as in the other species of the genus.

The mouth capsule is strongly developed, like that of *S. edentatus* it appears markedly cup-shaped when seen in a dorsal or ventral view (Fig. 1 A), in a lateral view (Fig. 1 B) the dorsal wall is seen to be shorter and more convex than the ventral, thus recalling the similar structure of *S. vulgaris*.

The dorsal gutter is well developed but considerably shorter than in the other species, its anterior termination being some distance behind the anterior margin of the mouth capsule (Fig. 1). A single tooth arises from the base of the dorsal gutter, like that of *S. vulgaris* it is divided into two broad lateral projections which are however relatively much lower than in that species and subdivided by ill-defined grooves into a number of rounded cusps. The latter are variable in number, in the majority of specimens, however, three principal cusps are conspicuous (Fig. 1 A), each composed of several smaller ones. There are no ventral teeth such as are found in *S. equinus*.

The excretory pore is situated in the head region as in *S. equinus* and *S. edentatus*.

Oesophagus: 1·6–2·4 mm. in length. The cervical papillae are in the neighbourhood of the nerve-ring, about 1·5 mm. from the anterior extremity of the body.

Female: 30–42 mm. in length, 1·8–2·5 mm. in extreme breadth. The head has a width of 0·9–1·3 mm.

Vulva 6–7 mm. from the posterior end of the body. The tail region is similar to that of *S. equinus* and *S. edentatus*; the anus is 0·4–0·6 mm. from the posterior extremity.

Male: 18–32 mm. in length, with a maximum breadth of about 1·8 mm. The head is 0·6–0·9 mm. broad.

As in the two larger of the known species of *Strongylus* the genital bursa is small as compared with the size of the body, it measures 1·1 in width in the largest specimen before me. The median lobe is short (Fig. 2).

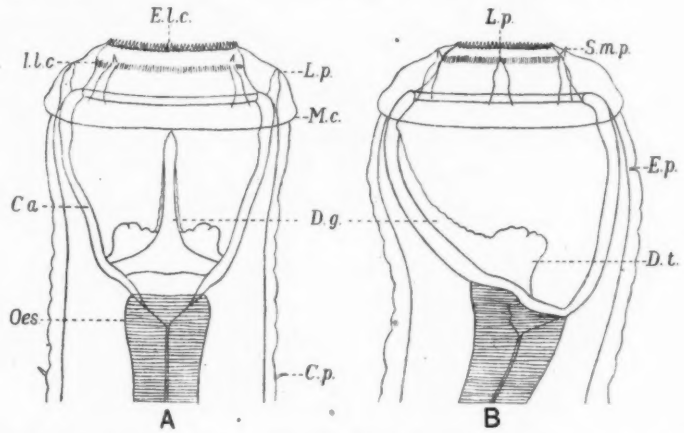

Fig. 1. *Strongylus asini* sp. n. A. Dorsal view of anterior extremity, × 37. B. Lateral view of anterior extremity of another specimen, × 37.

The rays of the bursa are similar to those of *S. edentatus*, *i.e.* they are more slender than those of *S. equinus*. The branches of the posterior rays differ from those of the other species in that the inner (dorsal) branch is simple, the external branch divided, *i.e.* the converse of the arrangement prevailing in the genus. This character may however not be specific. I was able to examine three male specimens only and the mode of branching of the posterior rays is liable to variation in many species of Strongyles. The spicules measure approximately 1·6 mm. in length.

Habitat: (1) Zanzibar, in a cyst of the liver and in the caecum of the donkey. (2) Nairobi, East Africa, in the caecum of the donkey.

The Zanzibar specimens consisted of three females and one male (all fully developed) from the liver cyst, whilst another male was found among a number of specimens of *S. edentatus* and *S. vulgaris* from the caecum. The Nairobi

collection contained one male in a good state of preservation, whilst a number of others occurred in material which had evidently been dry at some period, the worms being greatly shrivelled and full of air-bubbles; the species of *Strongylus* could only be identified by dissecting out the mouth capsule after maceration with Eau de Javelle. The specific diagnosis is therefore based on six specimens only (3 ♂♂ and 3 ♀♀).

The occurrence of fully developed specimens of this form in the liver is a point of interest, immature specimens of *S. equinus* have been observed in this situation but are more commonly met with in the pancreas; stages of this species and of *S. edentatus* have also been found in the hepatic ligament (Railliet and Henry 1902, Railliet 1915).

Fig. 2. *Strongylus asini* sp. n. Lateral view of male bursa, × 37.

Genus Cylicostomum Railliet and Henry[1]

(= *Cylichnostomum* Looss).

Cylicostomum adersi sp. n.

A single male specimen of this worm was found in Mr Aders' collection; the specific diagnosis is therefore based on this sex only.

Specific diagnosis. *Cylicostomum*: A large species presenting much the same appearance as *C. insigne* Boulenger (1917).

Head marked off from the body by a slight neck-like constriction. The mouth collar is comparatively narrow (Fig. 3).

The lateral head-papillae do not project from the surface of the collar, submedian papillae short and leaf-shaped.

The external leaf-crown consists of about 26 large pointed leaves surrounding the circular mouth-opening, the internal leaf-crown of double this number of similar but less conspicuous elements.

[1] In a recent paper (1917) I used Looss' generic name for these worms, there seems however no doubt that the form *Cylicostomum* has priority.

The mouth capsule is broad, having a depth of 70μ with a breadth of 140μ. The wall of the capsule is increased posteriorly to form a very well-marked "hoop-like" thickening (Fig. 3).

A dorsal gutter is present but is very short, just projecting into the mouth capsule. There is a well-developed oesophageal funnel (Fig. 3).

Male: 14 mm. in length, with a maximum breadth about ·75 mm. near the middle of the body. The head is nearly 200μ broad.

The oesophagus has a length of 700μ; it reaches its maximum breadth

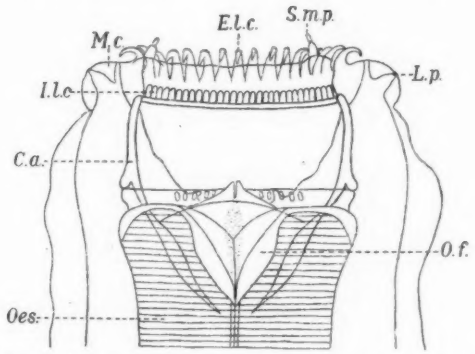

Fig. 3. *Cylicostomum adersi* sp. n. Anterior extremity in dorsal view, × 110.

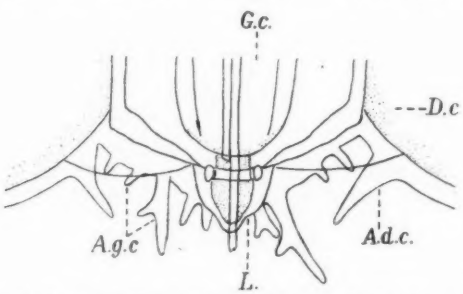

Fig. 4. *Cylicostomum adersi* sp. n. Ventral view of the genital cone to show the appendages, × 280.

(170μ) about the middle of its posterior half. Cervical papillae are situated 650μ from the anterior extremity of the body.

The bursa (Fig. 5) has a broad median lobe of moderate length. The comparatively short genital cone is completely surrounded by a well-developed dermal collar. Prebursal papillae slender and very long (250μ).

The appendages of the genital cone are completely fused in the middle line, forming a thin semicircular plate the margin of which bears four pairs of delicate finger-shaped processes, some of which have bifurcated extremities. On each side of this plate is an additional process arising from the dermal collar (Fig. 4).

Habitat: Zanzibar, caecum of donkey.

This species is evidently closely allied to *C. insigne* and belongs to the group of species which includes this form and *C. elongatum*, *C. radiatum* and *C. auriculatum*. It is distinguishable from *C. insigne* by the character of the genital appendages, by the number and shape of the leaves of the internal leaf-crown, and the presence of a highly chitinized oesophageal funnel.

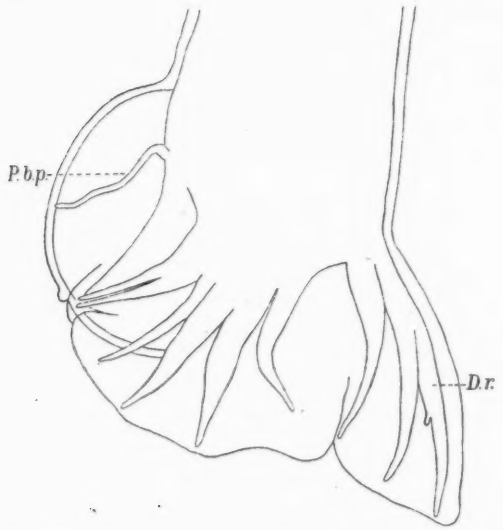

Fig. 5. *Cylicostomum adersi* sp. n. Lateral view of male bursa, × 75.

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EXPLANATION OF LETTERING.

A.d.c.	Appendage of the dermal collar.	*G.c.*	Genital cone.
A.g.c.	Appendage of the genital cone.	*I.l.c.*	Internal leaf-crown.
Ca.	Wall of mouth capsule.	*L.*	Ventral lip of the genital cone.
C.p.	Cervical papilla.	*L.p.*	Lateral head-papilla.
D.c.	Dermal collar of the genital cone	*M.c.*	Mouth collar.
D.g.	Dorsal gutter.	*Oes.*	Oesophagus.
D.r.	Dorsal ray of the bursa.	*O.f.*	Oesophageal funnel.
D.t.	Dorsal tooth.	*P.b.p.*	Prebursal papilla.
E.p.	Excretory pore.	*Sp.*	Spicule.
E.l.c.	External leaf-crown.	*S.m.p.*	Submedian head-papilla.

OBSERVATIONS ON BILHARZIASIS AMONGST THE EGYPTIAN EXPEDITIONARY FORCE.

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(With Plates III–V and 3 Text-figures.)

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I. HISTORICAL.

In 1851, Bilharz (1852) first discovered paired adult trematode worms originally named after him in the portal system of an Egyptian fellah. Subsequently, investigations of the deposits of ova in excreta were made, but numerous attempts by Cobbold, Sonsino, Lortet and Vialleton (1894) and others, to unravel the life history of the parasite, failed. Looss (1896), appreciating that the digenetic trematodes must necessarily pass through a molluscan intermediary, dissected many species of snails collected from the fresh-water canals around Cairo. He failed to find the cercariae of bilharzia, and discarded the hypothesis of an intermediate molluscan host. As, however, the miracidium of *Schistosomum*

haematobium contained germinal cells within its body cavity, it must, naturally, he argued, have been destined to produce sporocysts at some stage of its life cycle. In view of this, Looss evolved the hypothesis that man acted simultaneously as the intermediary, as well as the definitive host of this parasite.

According to his hypothesis the miracidia after reaching water perforated directly the skin of man, thence making their way to the liver. Here they developed into male and female sporocysts which in turn gave rise to male and female cercariae, and these, in their turn, developed into adult worms. Manson on the basis of biological analogy always opposed this view.

Looss also denied the existence of two species of bilharzial parasites, basing his contention on the absence of constant morphological differences in the two hypothetical species. He regarded the lateral spined ovum as characteristic of the unfertilized, and the terminal spined ovum as the one associated with the fertilized female; further he maintained that he had seen both varieties of ova simultaneously in the uterus of the same female worm.

Sambon (1907) supporting the views of Manson (1903), and Sonsino, wrote an able paper establishing the identity of a new species of bilharzia which he named *Schistosomum mansoni*. He based his reasoning on the different geographical distribution of the two forms of infestation, on the different systems pathologically involved, and on the morphological differences in the ova of the two species. Looss bitterly opposed his reasoning.

Bour (1913) failed to convey the disease to monkeys by injecting miracidia intravenously.

Miyagawa (1912) described the entrance of the infesting form of *S. japonicum*, an allied species, through the skin of the host, to the portal system. Miyairi and Suzuki (1913) demonstrated that the fresh-water mollusc, since named *Blandfordia nosophora*, acted as the intermediate host of *Schistosomum japonicum*. These investigators also conveyed the disease to mice by placing them in water containing infested snails. Ogata (1914) accurately described the morphology of the cercaria of *S. japonicum*, and later, Leiper and Atkinson (1915) confirmed all these observations, in Japan. In 1915, Leiper came in charge of the Bilharzia Mission to Egypt. As a result of the work of this mission, the existence was established of two species of *Schistosoma* morphologically distinct and producing different ova. From these ova, miracidia are hatched, which developing in certain fresh-water molluscs, produce cercariae, which have slight morphological distinctions.

The transmission of the disease to laboratory animals was effected and the affinity of *S. haematobium* for the vesical, and *S. mansoni* for the alimentary systems demonstrated.

In 1915–16, Cawston, in South Africa, showed that a fresh-water mollusc *Physopsis africana* (a near relative of *Bullinus*) acts as the intermediary host of *S. haematobium* in that country, and, together with Becker, claims to have produced artificial infestation of this species of snail with miracidia

hatched from terminal spined ova, and has described the morphology of the cercariae so produced.

Lutz (1917) and Iturbe (1917) have confirmed Leiper's observations in Brazil and Venezuela respectively by tracing the development of *Schistosomum mansoni* in the local species of *Planorbis* (*P. olivaceus*), and the former has succeeded in infesting rabbits and guinea-pigs under experimental conditions.

It is only fair to state that after the Japanese discoveries were published, Looss, still mindful of his own failure and that of Sonsino to identify bilharzia cercariae amongst the numerous developmental forms found in snails in the endemic centres of Egypt, suspected that *Schistosomum haematobium* had a life history which materially differed from *S. japonicum*.

In estimating Leiper's work on this subject one should not forget that was the prevalent view at the beginning of the war, and furthermore the real value of much of the Japanese work was lost to science at that time because the original articles were printed in Japanese journals in the native language and had not then been translated into either German or English.

II. SCOPE OF THE PRESENT INVESTIGATION.

The experimental data and observations on which this paper is based were collected after the departure of the Bilharzia Mission from Egypt at different periods, as far as military exigencies would permit, from 1916 onwards till the end of 1918. Through the generosity of the Australian Red Cross Society and of the Australian Military authorities, the cost of the experimental monkeys which were utilized was defrayed.

During these years a large number of troops, Imperial, Colonial and Indian, were stationed in districts heavily infested with bilharziasis. Although timely warnings were given by Dr Leiper and his Commission, a considerable number of troops became infested. From the fact that isolated posts were maintained during 1916 and part of 1917 along the Sweet-water Canal, and large bodies of troops were stationed in the Fayoum in close proximity to various collections of water it is not surprising that many men became victims of the disease.

It is probable that we have been able to trace but a small percentage of the infections originally acquired in Egypt, as a large number of men who had been stationed in the endemic zones were moved to France during the years 1916 and 1917 and probably only developed the symptoms of the disease at a later period.

The earliest cases among the Australian troops were accompanied by pyrexia, urticaria and general toxaemia and thus escaped recognition at first sight. These were probably examples of a massive infection; in the later cases, especially the more chronic vesical infections, these generalized symptoms were not so common.

The results of the present enquiry, which has entailed a considerable amount of work and the dissection of over 10,000 snails, has been to confirm

in a remarkable manner the historic investigations of Leiper, Thompson and Cockin, both as regards the duality of the species of Schistosomidae, and as regards their selective affinity for the two genera of fresh-water molluscs.

The data which have been obtained from the accidental infestation of the troops in different parts of Egypt in association with the local snail fauna amply confirm those obtained from experimental animals.

In addition, certain observations were made on the distribution of the different species of snails and their habits which have a bearing upon the prophylaxis of the disease, and it is with this end in view, and also with the idea of promulgating further investigations on the general prophylaxis of bilharziasis, that we submit this paper for publication.

III. THE MORPHOLOGY AND LIFE CYCLE OF THE TWO SPECIES OF EGYPTIAN SCHISTOSOMIDAE.

(*Schistosomum haematobium*—Bilharz v. Siebold, 1852.)
(*Schistosomum mansoni*—Sambon, 1907.)

In *Schistosomum haematobium* infestations terminal spined ova are deposited in the excreta, both urine and faeces, of an infested subject. On reaching water a ciliated miracidium is hatched out, which in a period of thirty-six hours at a maximum must find the correct species of snail of the genus Bullinus, failing which it dies. If a suitable snail is encountered, the miracidium enters it by piercing its soft parts, probably through the pulmonary chamber, and eventually reaches the digestive and the hermaphrodite glands. Here it is converted into a morula and later becomes hollowed out into elongated finger-like sporocysts; these, by a process of budding of cell masses known as "germ balls" or blastospheres, produce cercariae; they may also by a process of exogenous budding produce secondary cysts. When mature, the cercariae are ejected into water, and in the subsequent twenty-four hours must meet their definitive host, usually man, and after invading his tissues, either viâ the skin or the upper alimentary tract, make their way to the portal veins in which they develop into adult worms, both male and female.

In *Schistosomum mansoni* infestations lateral spined ova are excreted in the faeces of an infested subject, less commonly in the urine as well. The intermediate host in this case also is a fresh-water snail, but of the genus *Planorbis*. In other respects the life cycle resembles that described above for *S. haematobium*.

The Ova.

The ova of the various species of schistosomes affecting man are passed to the exterior in the excreta, and in this fashion reach water. These ova are of a dark yellow brown colour, and of different shape; they are non-operculated, but possess either a spine or the rudiments of one. The ovum is covered by a chitinous shell, lined with a thin shell membrane, its interior

being practically filled by a fully developed ciliated miracidium (Plate III, figs. 1 and 2).

The following table, Table I, will serve to illustrate the main difference in the ova of the three species of Schistosoma.

Table I.

Differences in the Ova of the various Schistosomidae affecting man.

Schistosomum japonicum (Katsurada, 1904) vel. *S. cattoi* (Blanchard)	*Schistosomum mansoni* (Sambon, 1907)	*Schistosomum haematobium* (Bilh. v. Sieb. 1852)
(1) *Ovum* 70–75 μ long, 45–55 μ broad	*Ovum* closely resembles *S. haematobium*, but is on the average slightly shorter	*Ovum* 120–160 μ long, 40–60 μ wide
(2) *Shape* more rounded than other species	*Shape* Oval or spindle-shaped	*Shape* Oval or spindle-shaped
(3) *Spine.* No spine but a rudimentary lateral papilla	*Spine.* Lateral spine	*Spine.* Terminal spine
(4) *Excreted* in faeces, not in urine	*Excreted* in faeces, less frequently in urine	*Excreted* in urine and faeces

HATCHING OF THE OVA.

The ova may be readily hatched out by lowering the salt content of the fluid which bathes them, this occurs best at a temperature of 100° F. Microscopically, the first change noted under these conditions is a movement in the lateral walls of the ovum, at the point of its greatest transverse diameter. Activity of the cilia covering the miracidium is then noted and fluid currents appear to be set up within the shell membrane. This actual process, we believe, is due to the chitinous envelope and shell membrane acting conjointly as a semipermeable membrane, with the resultant passage of water from the fluid of lower to one of higher osmotic pressure. This ingress of water swells out the spindle-shaped ovum and increases its transverse diameter, and the miracidium, stimulated by the change in its environment, starts its ciliated activity. These two factors result in rupture of the chitinous shell in a lateral direction, as a general rule, slightly posterior to its central transverse axis.

Occasionally free miracidia may be found in freshly voided urine or faeces, but such an occurrence is very exceptional. After rupture of the chitinous envelope the miracidium still enveloped in the shell membrane may be seen partially extruded through the opening thus produced. Rupture of the membrane generally occurs during this process of extrusion, and is probably due to its tearing on the jagged chitinous edges. Once freedom is attained the miracidium swims rapidly away on its adventurous quest.

MIRACIDIA.

A limit of 36 hours is set down in which the miracidium must meet its specific intermediate host. In structure it closely resembles the miracidium of *Fasciola hepatica*, the most marked difference being the absence of eye spots.

It is covered with cilia, except at the anterior end where a papilla is situated. Excretory organs, and round clear germinal cells are both noticeable in the body cavity, and two or three transverse constrictions may be noted (while the miracidium is still unhatched) as grooves or indentations of the lateral borders (Plate I, figs. 1 and 2). On reaching its specific intermediate host, the previously enumerated species of *Bullinus* snail in the case of *Schistosomum haematobium*, and in the case of *Schistosomum mansoni*, *Planorbis boissyi*, the miracidium gains entrance by boring through the soft tissues, and eventually reaches the digestive gland, or liver.

Here, as previously noted, by proliferation of its germinal cells, the mother and daughter sporocysts are formed, and the ultimate development of the cercariae takes place in their interior.

A sporocyst is a simple sac, without either alimentary canal or oral sucker. The sporocysts under consideration are elongated finger-like cysts with thin walls, thickened at either end. They have independent but feeble power of movement, and appear to absorb nourishment directly through their walls. Over distension of the daughter cysts leads to rupture, and the evacuation of their contents. Where rupture takes place prematurely, we noted the following developmental forms:

(1) Rounded or oval masses of cells.

(2) Elongated oval masses of cells showing a tendency to form a posterior process.

(3) A shapeless type of cercaria, with a partially developed anterior part, and a blunt posterior part—the future tail. The earliest indication of the forked nature of the latter is seen in two small buds at its posterior end.

(4) The fully developed mature cercaria with anterior and lateral suckers, and a long bifid tail.

The above enumerated forms evidently represent the different stages in the development of the cercaria from the germinal cells of the sporocyst. The enormous development which may take place is shown in Plate V, fig. 1, where almost all the normal glandular tissue is displaced by sporocysts.

Morphology of Cercariae of *S. haematobium* and *S. mansoni*.

The specimens for this purpose were obtained by dissection of the livers of infected snails of the species, *Planorbis boissyi*, and *Bullinus contortus* and *dybowski*.

These organs were teased out and the contained cercariae killed by heat. The specimens were then examined under a cover-glass. No marked difference was noted in their internal structure from the description given by Leiper in his original paper on the subject and the more recent minute one by Faust (1919). In live specimens the movements also correspond. In the main points of structure they resemble the cercaria of *S. japonicum*, as described by Ogata (*Verh. der Japn. path. Gesellsch.* Tokyo, 1914, vol. 48).

All Schistosoma cercariae, whether of the species *S. haematobium*, *S. mansoni*, or *S. japonicum*, belong to the furcocerous group of the Distome cercariae: Leiper emphasizes the following common characteristics of the Bilharzia group of cercariae.

(1) The absence of the pharynx.

(2) The presence of a forked or bifid tail.

(3) The presence of anterior and ventral suckers.

(4) The absence of eye spots.

(5) The presence of two or more sets of glands situated posteriorly, one on each side of the body, and communicating with the mouth.

The following measurements for the cercariae of *S. mansoni* were made from 40, and those of *S. haematobium* from 35, freshly dissected specimens. The measurements are as tabulated in Table II below.

Table II.

Summary of Measurements.

	Column I Cercariae of *S. japonicum* (Leiper)	Column II Cercariae of *S. mansoni*	Column III Cercariae of *S. haematobium*
1.	Total length of *body and tail* 0·25 mm.	Total length of *body and tail* shorter than *haematobium* 0·374 mm.	Total length of *body and tail*, longer 0·398 mm.
2.	Body 0·1 mm.	Body, slightly shorter 0·161 mm.	*Body*, slightly longer 0·190 mm.
3.	Breadth of body 0·04 mm.	Breadth of body 0·060 mm.	Breadth of body 0·064 mm.
4.	Post prolongation of *oral sucker* from anterior extremity 0·04 mm.	Posterior prolongation of *oral sucker*, from anterior extremity 0·053 mm.	Posterior prolongation of oral sucker, from anterior extremity 0·073 mm.
5.	Ventral sucker	*Ventral sucker*, from posterior extremity 0·044 mm	*Ventral sucker*, from posterior extremity 0·049 mm.
6.	Length of *tail to bifurcation* 0·1 mm.	Length of *tail to bifurcation*, longer 0·213 mm.	Length of *tail to bifurcation*, shorter 0·208 mm.
7.	Breadth of *tail* 0·01 mm.	Breadth of *tail* 0·035 mm.	Breadth of *tail* 0·031 mm.
8.	Length of each arm of fork 0·005 mm.	Length of *each arm of fork*, shorter 0·066 mm. × 0·008 mm.	Length of *each arm of fork*, longer 0·081 mm. × 0·008 mm.
9.	Ventral sucker slightly protuberant	No marked protrusion of ventral sucker on lateral view (*vide* Plate III, fig. 3 *a*)	Marked protrusion of ventral sucker on lateral view (*vide* Plate III, fig. 4 *a*)

Our measurements for the cercaria of *S. mansoni* are larger than those given by Faust, Iturbe and Gonzalez, while our measurements of *S. haematobium* cercaria are slightly smaller than the latest given by Faust (June, 1919). Regarding the details, see Plate III, figs. 3 and 4.

The anterior sucker appears to be provided with a number of papillae (about 16), which can be protruded or retracted by muscular action, and which

probably assist the cercaria to cling to any stationary object, and in this way to gain entrance through the skin of its host. Lutz (1917) has noted on the head of the cercariae a ring of six or more small points, like the crown of a trephine, which may be considered to be a boring organ. This feature is not noted by Leiper, but the Japanese authors observed it in *S. japonicum* without attaching any importance to it. The oral sucker has a posterior prolongation (pharyngeal pouch), into which the ducts of the poison or salivary-mucin glands open.

The ventral sucker or acetabulum is circular in outline and lies in the posterior fifth of the body, and when the cercaria is at rest the opening of this sucker is tri-radiate in shape. Lying immediately in front of the ventral sucker there is a small round brown pigmented simple gland of a granular consistency from which a small duct runs forward into the mouth. This can only be made out in fresh, but not in stained, specimens.

On each side of this lie the "poison cells" or salivary glands, each of which contains a well-marked nucleus, and from these individually a duct can be distinguished passing forward into the mouth. The number of these salivary-mucin glands varies in the two species, as pointed out by Faust, and which we have been able to confirm from our specimens. In *S. mansoni* cercaria (Pl. III, fig. 3) there are six pairs of glands altogether, of which two are large and clear with large nuclei and four are small and granular with correspondingly smaller nuclei. In *S. haematobium* cercaria (Pl. III, fig. 4) there are three pairs of large clear cells with acidophil protoplasm and clear cut nuclei. In front of the salivary glands the primitive nerve ganglion divides into two branches; it would appear that this structure has been mistaken for the oesophagus.

Immediately behind the ventral sucker there is a collection of densely packed nuclei, representing, probably, a primitive genital centre, and laterally placed to these are two oval excretory flame cells. The junction of the body and the tail is delimited by a membrane of oval shape and delicate consistency. The diameter of the tail diminishes towards the fork. Each arm of this fork tapers towards its termination and ends in a definite spike. The integument of the body as well as of the tail is covered with minute spines which can only be made out in the more mature cercariae, as has been described by Leiper for *S. japonicum* cercaria.

The Excretory System.

The excretory, or protonephridial system of these schistosome cercariae appears to be identical and has been described by Cort. It consists of six pairs of flame cells arranged along the margins of the body; from these cells capillary canals arise which join collecting tubules of a greater calibre running both in an anterior and a posterior direction.

The excretory bladder consists of symmetrical convoluted tubes which project in a V-shaped manner just in front of the acetabulum (or posterior sucker), thence they run backwards and fuse at the junction of the tail to form

a single tube which extends to the posterior extremity; bifurcates once more, and opens on the tip of each bifurcation.

The *deportment* of the cercaria appears to be as follows: it generally swims with the expanded tail uppermost, and may be seen resting on the surface with the forks spread almost at right angles to the rest of the tail. When many cercariae are present in a sample of water they arrange themselves at regular intervals from one another throughout the different strata. Should the surface of the water then be disturbed, the cercariae immediately become active, and by vibratile motions of the tail make their way to the point from which the disturbance commenced.

Oxygen is necessary for their existence, and for this reason cercariae put up in hanging drop preparations in an air-tight vaseline compartment die with much more rapidity than those kept for a similar time in open glass jars.

The cercariae of *S. japonicum*, according to Leiper and Faust, are of a smaller size than those of *S. haematobium* and *mansoni*, and as in the latter species the whole body is covered with minute spines. The oral sucker is enormously developed, occupying almost the anterior third of the body. At the lip of the sucker there is a series of small tubercles and, according to Faust, the salivary-mucin glands consist of five pairs with acidophilic protoplasm arrayed around the ventral sucker.

MODE OF ENTRY INTO HOST.

We already know that once ejected from the intermediate host, the cercaria has but from 24[1] to 36 hours in which to come into contact with the skin, or mucous membrane of the upper alimentary tract of man, its definitive host.

The actual method by which penetration of the skin or mucous membrane takes place is still under investigation. Two factors are, however, probably concerned:

(1) The secretion by the cercaria of some chemical substance which has the property of dissolving the inter-cellular substance between the epithelial cells.

(2) The power which the cercaria possesses of rapidly altering its shape by contraction or relaxation of its body.

Leiper demonstrated the process by immersing a mouse for half an hour in water heavily infected with cercariae, and subsequently embedded it in paraffin. Microscopically, sections showed cercariae in various stages of transit through the unbroken skin. At the end of the half hour only a few of the cercariae were left in the water, but a great number of detached tails were observed. Warmth would appear to be the main factor in attracting the cercariae to the skin.

[1] In actual practice during the summer months in Cairo we found that the majority died after 12 hours.

After the cercaria adhered to a fixed object, such as the skin of its host, by means of the oral sucker, the tail is cast off from the body in the following manner. The mouth muscles are contracted, and this act is followed by a protrusion of the papillae of the oral sucker; simultaneously, waves of contraction pass along the body and the ventral sucker also obtains a firm hold of its victim.

Rapid vibratile side-to-side movements of the tail then take place, and the fork is opened and closed in a forceps-like manner. In a short space of time the tail parts from the body at its membranous junction and floats away.

Artificial Infestation of Monkeys.

During the present investigation, we have produced artificial schistosomiasis in 24 monkeys.

4 were infested with *Schistosomum haematobium*.

20 were infested with *Schistosomum mansoni*.

In most cases the dual routes of infestation, *i.e.* viâ both the skin and upper alimentary tract, were employed, but in two, infestation was produced by infected drinking water, and in six others, by the skin route only.

Marked pruritus was a prominent manifestation in these monkeys; they scratched their skin or lips on those portions which had come in contact with the infected water. Many of the soldiers infected at Tel-el-Kebir gave such a history of itching while bathing, or immediately after leaving the water. Indeed some of them actually left off swimming on this account. In three of the monkeys, definite skin rashes were observed the day after exposure, on the areas exposed to the action of the infected water. The existence of a similar rash in *S. japonicum* has been noted by Miyagawa.

Similar phenomena were observed at the village of el Marg on the arms and legs of the Arab boys who collected snails from that locality, after immersion in the water of the smaller canals during collection of specimens. In the most heavily infected zones the itchiness was so marked that sometimes the boys voluntarily left the water on that account. In these cases, even within as short a period as 20 minutes, whitish papular elevations, the size of a small pin's head, appeared closely together over the skin of their extremities. This rash never persisted for more than 48 hours. Intense itchiness was present, and scratching sometimes led to reddening of the part due to extravasation of blood into the papules. Infested snails could always be demonstrated in large numbers in water that produced these symptoms. As a matter of fact, we were able to learn the position of several highly infected localities, by finding, from interrogating the Arab collectors, those places where they developed this pruritus during collecting.

In infestations through the upper alimentary tract the cercariae attach themselves to the mucosa of the buccal cavity either of the pharynx or of the oesophagus.

A solution of hydrochloric acid, 1 in 1000, was found to quickly kill the

most active cercariae, so it may safely be assumed that they die on reaching the stomach.

After penetrating the skin or mucosa, the cercariae are conveyed by the venous system to the right heart and so to the lungs. Here they may conceivably be temporarily held up in the pulmonary capillary bed, but eventually reach the portal vein and liver.

The lung of a monkey exposed to heavy infection three days previously showed no cercariae on section.

Once having reached the portal venules, the cercariae develop into adult male and female worms; the latter being then enveloped in the gynaecophoric canal of the former. Subsequently the paired worms travel against the portal blood stream to their ultimate destinations.

The different distribution of the paired worms in the two species of infestations is of considerable interest. In *S. mansoni* infestations, the habitat of the worm in infested monkeys was the inferior and superior mesenteric veins, and the portal veins of the liver.

In *S. haematobium* infestations, paired worms were found in portal veins of the liver, as well as in the inferior and superior mesenteric veins, *i.e.* the vesical and uterine branches. From here many wandered into the inferior vena cava, and were filtered out into the lung. This tendency was never noted in *S. mansoni*, though their lateral spined eggs have been found in those situations.

MORPHOLOGY OF ADULT PARASITES.

General characteristics common to both species of worms.

The *male schistosomes* are 1–1·5 cm. in length. The greatest breadth posterior to the ventral sucker is 1 mm. The anterior truncated portion is short, narrow and cylindrical, and bears suckers; the posterior part is flat and leaf-like; more posteriorly the lateral margins of this fold inwards and overlap, forming the gynaecophoric canal in which the female lies. The ventral sucker is situated just anterior to the junction of the fold. Posterior to the ventral sucker, the body of the male is covered with wart-like projections. The suckers are two in number, and are covered with papillae. The oral sucker is situated anteriorly and is funnel-shaped, with the dorsal lip larger than the ventral and communicating with the mouth.

The ventral sucker is longer than the oral, is somewhat triangular in shape, and the apex of the triangle forms the stalk of the attachment.

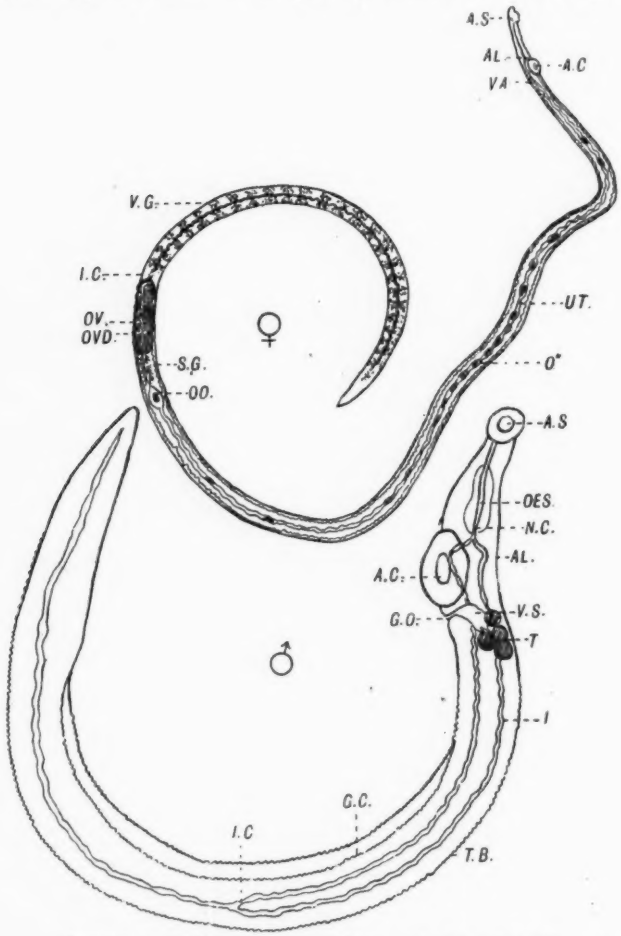
The function of this particular sucker would appear to be to fix the worm in a stable position while that of the oral sucker is essentially prehensile and alimentary.

The excretory pore is placed posteriorly and is dorsal in position. The genital opening lies just posterior to the ventral sucker in the median line.

The alimentary canal presents a mouth opening into the oral sucker and a short straight oesophagus which bifurcates just posteriorly to the ventral

sucker, and ultimately gives rise to the intestinal caeca. These caeca pass back laterally when they fuse to form a single tube which terminates posteriorly in a blind end.

The intestinal caeca are dark in colour owing to digested blood. The reproductive organs consist of irregularly rounded testes lying dorsal to



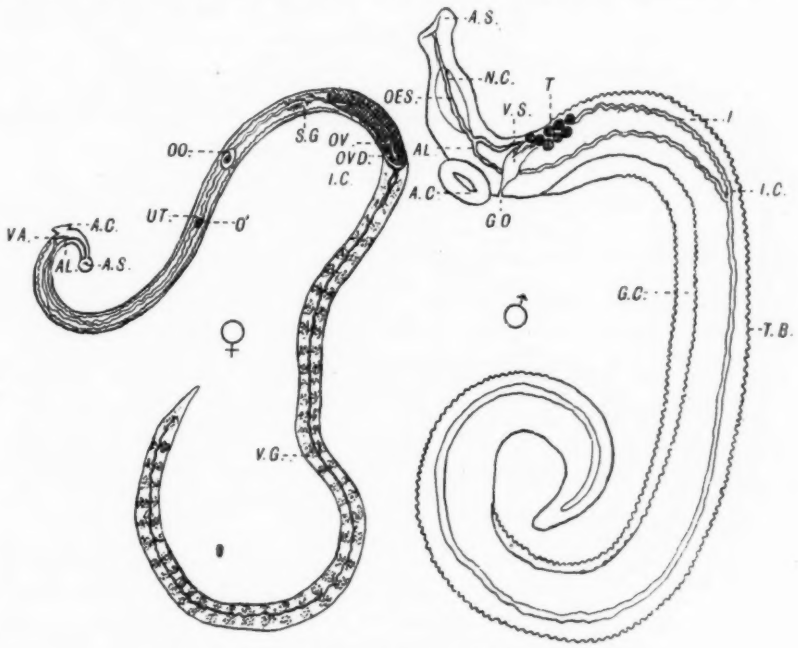
Text-fig. 1. *Schistosomum haematobium*. Adult ♂ and ♀ × 30.
From drawings made to scale by Dr J. K. Lund.

LETTERING TO TEXT-FIGURES 1 AND 2.

A.S. anterior sucker; *A.C.* ventral sucker; *AL.* bifurcation of alimentary canal; *OES.* oesophagus; *N.C.* nerve chord; *G.O.* genital opening; *V.S.* vesicula seminalis; *T.* testes; *I.* intestine; *I.C.* union of intestinal caeca; *G.C.* gynaecophoric canal; *T.B.* tuberculations; *VA.* vagina; *UT.* uterus; *O'* lateral spined ovum; *O"* terminal spined ovum; *OO.* ootype; *S.G.* shell gland; *OV.* ovary; *OVD.* oviduct; *V.G.* vitelline glands.

the ventral sucker. Vasa efferentia lead from each testis and unite to form the vesicula seminalis. This is an elongated tube which narrows anteriorly and passes into the vas deferens, and this in turn opens on to the exterior by the genital pore by means of an ejaculatory duct.

The *female schistosomes* are cylindrical and thread-like, 1·5 to 2 cm. in length, and tapering at the anterior and posterior extremities. In breadth they are a quarter that of the male—0·25 mm. The body is smooth save towards the posterior end and on the suckers where there are papillae. They possess two suckers, oral and ventral. The alimentary canal is similar to that



Text-fig. 2. *Schistosomum mansoni*. Adult ♂ and ♀ × 30.
From drawings made to scale by Dr J. K. Lund.

of the male, the caeca uniting to form a single median tube zig-zag in appearance.

The *reproductive organs* consist of (1) an ovary, an elongated oval body lying anterior to the re-union of the intestinal caeca. The oviduct arises from its posterior end and bends forward to unite, anteriorly to the ovary, with the vitelline ducts from the yolk gland. (2) The yolk glands which occupy the posterior part of the body, lateral to the single tube formed by the intestinal caeca. (3) The shell gland which also opens near the junction of the vitelline ducts and the oviduct. (4) The uterus which is a long tube opening on to the exterior at the genital pore.

The difference in the two species *Schistosomum haematobium* and *Schistosomum mansoni* is enumerated in detail in Table III, which has been constructed after a careful study of the morphology of these two schistosomes.

Table III.

Differences observed in the two species of Schistosomum. (Text-figs. 1 and 2.)

S. haematobium (Bilh. v. Sieb. 1852). (Fig. 1)	*S. mansoni* (Sambon, 1907). (Fig. 2)
MALE	MALE
Length 1·5 cm.	On the average slightly shorter than *S. haematobium*, about 1 cm.
Finely tuberculated.	Grossly tuberculated.
Testes large and generally four in number, posterior to ventral sucker.	Testes small and eight in number, posterior to ventral sucker. Ventral sucker more prominent.
FEMALE	FEMALE
Length 2 cm.	Length 1·5 cm. maximum.
Ovary in posterior third of worm, in front of intestinal caeca.	Ovary in anterior half of body, in front of union of intestinal caeca.
Uterus contains large numbers of terminal spined ova with spine directed backwards (maximum number 50).	Uterus contains usually one, at most a few, lateral spined ova, with spine directed backwards.
Lateral branches of intestinal canal unite in posterior third.	The lateral branches of the intestinal canal unite in the anterior half of the worm.
Yolk glands distributed in posterior fourth of the body.	The yolk glands are widely distributed in posterior half of the body.

NOTE. From available literature it appears that worms of *S. japonicum* are of smaller size, the male being from 0·9 to 1·2 cm. long, and the female 1·2 cm. long, though Katsurada (1913) has described specimens 2 cm. and over. The ventral, relative to the oral sucker, is proportionally larger. The integument of the male is smooth, and non-tuberculated. The posterior part of the body is relatively wider, the over-lapping of the gynaecophoric canal being more extensive than in the other schistosomes.

THE HABITAT OF THE ADULT WORMS IN THE VEINS, AND THE METHOD OF DEPOSITION OF OVA IN THE TISSUES.

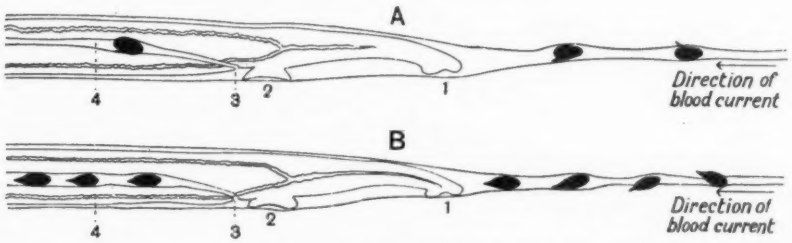
These observations were made on the adults of *S. mansoni* and *S. haematobium* lying in the mesenteric veins of anaesthetized monkeys.

The coupled worms, males and females, lying in the loops of the mesenteric veins, make their way to the finer venules, progressing by means of peristaltic movements of the body, and by action of the ventral sucker. The male assumes the chief part in this progress, the female being carried in a purely mechanical manner, in the gynaecophoric canal. Quite often, bunches of six or more coupled worms are seen progressing in one large vein in this manner.

The male adheres to the vessel wall, first by its ventral sucker, then by muscular contraction, the head lengthens out and a new grasp is obtained of the vessel wall. The anterior sucker does not appear to be used as an organ

of progression, but as an organ with an alimentary function. Where the worms do not entirely block the vessel, it is possible that their tuberculated skin enables them to maintain a stationary position against the blood stream. When desirous of depositing ova, the female protrudes herself from inside the gynaecophoric canal, and migrates in the manner described above, until she reaches a very fine branch. It is obvious that her smaller diameter enables her to do this with comparative ease, and when the diameter of the vein limits her progress she ejects her ova through her genital aperture, as shown in the diagram (Text-fig. 3, A and B). In the case of the *S. mansoni* these are few in number, but in the case of *S. haematobium* they are much more numerous. The greatest number of lateral spined ova noted in a venule has been six, whereas fully twenty terminal spined ova may occur actually jammed into one vessel. In each case the spine is directed towards the larger diameter of the vessel, *i.e.* in the direction of the flow of the portal blood.

Now the transverse diameter of the anterior truncated part of the female



Text-fig. 3. Diagram representing method of deposition of ova into the lumen of a small vein by the female and their subsequent passage through the vessel wall: **A** in the case of *S. mansoni*, and **B** in the case of *S. haematobium*.

1. Anterior sucker. 2. Posterior sucker. 3. Vaginal orifice. 4. Uterus with contained ova.

worm when stretched out in this manner becomes not very much greater than that of the deposited ovum, therefore it follows that these eggs must be in very close apposition to the walls of the vessels. By blocking the vessel in this manner the blood current in the vein, which has become stretched to accommodate the worm, becomes arrested.

When the female worm withdraws from the smaller branch vessel into which she has worked her way, towards one of larger diameter where she has left her male partner, the vessel wall immediately contracts down to its normal size and thus comes into contact with the deposited ovum; in consequence, there is every probability that the spine of the ovum will engage the vessel walls. Reflex spasm of the muscular tissue of the wall is an additional factor for ensuring this result. Apparently, after withdrawing a little, the female again becomes stationary; this act is again repeated, and in this manner a whole series of ova are deposited in small vessels giving the appearance of a string of sausages in miniature. The final withdrawal of the

female from a branch vein leads to a resumption of the blood current which impinging successively on the thin anterior ends of the ova drives them through the vessel wall into the perivenous tissues.

In the case of lateral spined ova, the marked increase in the transverse diameter of the ovum caused by presence of the lateral spine facilitates it in engaging the wall of the vessel (see Text-fig. 3).

In the case of the terminal spined ovum, unless its long axis lies mathematically parallel to that of the vessel, the impact of the blood current on its anterior end will turn it in an oblique direction. Immediately this occurs, the terminal spine will engage the vessel wall, and penetration will follow.

By these means the adult females reach the submucous tissue and the ova appear in the urine or faeces, as the case may be, within a few hours of being deposited, unless held up by fibrous tissue.

The description set forth above is not based on theory but upon the following observations:

(i) The female worm is often found separate from the male on opening the abdomen of an infested monkey under anaesthesia.

(ii) The female worms may be found singly in microscopic sections, filling up the veins of a calibre approximately equal to their own.

(iii) Direct microscopic examination of pieces of the intestine (of infested monkeys), squeezed between glass slides by means of rubber bands, will reveal the presence of ova in the vessels. Generally, several ova are deposited in a single vessel at close intervals. In many cases the diameter of this vessel is no larger than the transverse diameter of an ovum, and indeed, is often less, so that the vessel may present the appearance of a string of sausages. In some vessels ova could be observed lying in close apposition to the vessel walls, but without the spine having engaged. In others, the spines, both terminal and lateral, could be seen projecting through the vessel walls, while the bodies of the ova still occupied the lumen. In other cases ova could be observed lying free in the tissues outside the ruptured venule. The ova were always lying in the vessel with the anterior blunt end pointing in the direction of the decreasing diameter of the vessel, and with the spines in the direction of the portal blood current.

(iv) By means of a hand lens sacculations of the vessel walls could be observed corresponding with the long strings of ova referred to.

(v) Examination of the uterus of gravid females has revealed the ova *in situ*, and invariably they are placed with the spine, whether lateral or terminal, posteriorly, and the blunt narrow end of the ovum, anteriorly situated.

IV. OBSERVATIONS ON CERTAIN MOLLUSCAN INTERMEDIARIES, ESPECIALLY RELATING TO THOSE OF EGYPTIAN BILHARZIASIS.

PLANORBIS GROUP OF SNAILS.

(1) *Planorbis boissyi*, as its name implies, is quite flat when laid on an even surface. It acts as the intermediate host for *Schistosomum mansoni*. We have found two varieties of this snail at el Marg (near Cairo). One has a dark gray or black thickened shell, often covered with whitish incrustations, the other has a light brown thin shell free from any deposits. It is the latter variety that is most commonly infested.

This is the most common snail in the sweet-water canals at el Marg, and is to be found in many places in the Delta, at Ismailia, Tel-el-Kebir, Abou Soueir, and Tanta. Agglutinated masses may be found floating down stream in the canals, and in this way, snails originally infested in the more closely populated districts may be transported over wide stretches of country.

(2) *Planorbis mareoticus* was also met with at el Marg. Its chief characteristic was its small size.

BULLINUS GROUP OF SNAILS.

The differentiation of the four species of *Bullinus* and of their many transitional forms is no easy matter, except for the experienced conchologist. The four species are *Bullinus contortus*, *Bullinus dybowski*, *Bullinus innesi*, and *Bullinus forskali*. In common, they all possess a non-operculated sinistral shell, that is to say, the helix runs from left to right when the apex points upwards; and the mouth, facing the observer, is situated on the left. Leiper has shown that three of these species, *B. contortus*, *B. dybowski*, *B. innesi*, act as intermediary hosts for *S. haematobium*. The bodies of all these snails contain a red diffusible pigment.

The only other snail commonly seen in the Canal Zone with the above characteristics is *Physa subopaca*. It is easily distinguished by its rapid movement, by the different shape of the shell which is more pointed and elongated, by the different shape of the foot, and by the absence of any red pigment. Numerous attempts to infest the *Physa* artificially failed, and over 100 specimens collected under natural conditions, and dissected, were found to be free from cercariae. No attraction for this species of snail was exhibited by miracidia hatched out of terminal spined ova, though frequent experiments were made to investigate this point.

In colour, the *Bullinus* vary from greenish black to brown. If infested with *Schistosomum haematobium*, the apical convolutions are yellow when viewed by transmitted light. If infested with *Gastrodiscus aegyptius* (Cobbold), the apical whorl is an intense black, but if infested with a yellow coloured redia (probably *Cercaria lutea* (Gilchrist)), it is bright orange. Infestations with the two latter parasites are extremely common in these snails.

Observations on Infested Snails of *Bullinus* and *Planorbis* Genera.

Under laboratory conditions we have frequently found that infested snails die more rapidly than normal ones, even when great care is taken regarding feeding and supply of fresh water, etc. If out of a batch of such snails 100 are dissected at weekly intervals, the percentage of those infested is found to steadily decrease. Even under laboratory conditions however, and notwithstanding a heavy infestation, certain snails have been observed to produce cercariae for many weeks. It seems probable, therefore, under natural conditions that infested snails may survive for long periods.

We have observed very definite macroscopic and microscopic changes in infested snails.

The appearance of the infested snail. After a little experience the infested snail can be recognised by the naked eye, by certain stigmata; this more generally applies to *Planorbis*. The shell itself is glossy, smooth, and shiny in appearance, and is almost invariably friable—this point alone is a good indication of probable infestation.

The infested snails are, in the majority of cases, fully adult and the hermaphrodite gland contains ripe ova, though occasionally half-grown specimens have been found infested. In those heavily infested, the golden-yellow colour, so characteristic of the liver in this condition, may be seen appearing through the shell wall itself. The organ itself is swollen and friable; it may be either ochre, bright yellow, or greenish brown in colour, and abundantly speckled with gray spots. Generally the right lobe of the liver contains the greater number of cercariae, as does also the hermaphrodite gland, which lies midway between the two hepatic lobes. The fatty degeneration of the liver, as it would appear to be, is not brought about exclusively by the bilharzia cercariae, but occurs also in snails infested with the cercariae figured in Plate IV, fig. 5.

On microscopic examination deposits of sporocysts and cercariae in the interacinous tissue are to be noted. These displace the acini of the glands and cause pressure atrophy of the parenchyma; in heavily infected livers, fully 50 per cent. of the total mass consists of sporocysts and cercariae (Pl. V, fig. 1).

Confirmatory proof that cercariae obtained from Planorbis *develop only into adult worms having the morphology of* S. mansoni, *while those obtained from* Bullinus *develop only into adult worms having the morphology of* S. haematobium.

Monkeys were infested in the following way. A snail's liver, which was found microscopically to contain a large number of mature cercariae, was placed on a shaved area of the leg or abdomen of the monkey, and was permitted to remain in contact with the skin for from five to twenty minutes.

After an average period of six weeks, these monkeys became very thin and

debilitated, and were then chloroformed. The mature worms were then extracted in large numbers from the liver and mesenteric veins.

Both sexes of the worms were then found to be adult, the uterus of the females containing ova. Specimens were measured while alive, preserved by fixing in hot 70 per cent. alcohol, and then cleared in creosote. The morphological differences verified in this manner entirely corroborate those detailed by Leiper (1916) and on these observations Table III has been compiled.

Transmission experiments under artificial conditions with the object of determining whether Planorbis *acts as a strictly selective intermediate host for* S. mansoni *and* Bullinus (*Species* contortus *and* dybowski) *for* S. haematobium.

For this purpose experiments were undertaken in order to breed *Planorbis* in the laboratory, and thereby to ensure its freedom from previous infestation. This was found to be easily feasible, and a number of laboratory-bred snails were obtained from adults, which had been placed in a vessel three months previously. It was found that on an average, *Planorbis* took, in the atmosphere of Cairo in the spring and summer months, about three months to attain to maturity.

Thirty-six of these snails were placed in a vessel together with the centrifugalized urinary sediment containing ova of *S. haematobium*. On examination, two months later, a batch of nine were dissected and no cercariae were found. The remainder were then placed together with lateral spined ova of *S. mansoni*, occurring in the urine of an infested soldier. Subsequently after a further period of six weeks 21 per cent. of the survivors were found to be heavily infested with cercariae which gave measurements identical with those already recorded for *S. mansoni*.

Similar attempts to breed *Bullinus* in captivity were not attended with success, but a large supply of these snails was obtained in August, 1917, from a sedimentation tank, fed by filtered water from the sweet-water canal where there was no chance of faecal or urinary infection; in order to ensure that these snails were not infested by cercariae of any description, one hundred were dissected and found to be normal. Attempts were then made to infest a large number by means of lateral spined ova obtained from the urine with entirely negative results.

THE SEASONAL INCIDENCE OF INFECTIVITY OF *PLANORBIS* AND *BULLINUS* WITH THE TWO SPECIES OF *SCHISTOSOMIDAE*.

The accompanying tables show that considerable numbers of snails all obtained from the same locality (el Marg) were dissected for twelve consecutive months, and one remarkable fact emerges, namely, that the largest number harbouring cercariae of both forms occurred in the late autumn months, and especially in December. It was further noted that in these months

(late autumn) the livers of the infested snails harboured full grown cercariae, while in the early spring, immature cercariae and sporocysts were the rule rather than the exception.

Table IV.

Seasonal incidence in infestation.

Month	*Bullinus*		*Planorbis*	
	Number of snails dissected	Percentage infested	Number of snails dissected	Percentage infested
1917				
May	51	1·9	280	37
June	27	0·0	572	19
July	140	0·7	288	29
August	57	1·7	567	8
September	93	0·0	689	10
October	No record	No record	920	6
November	50	4·0	1468	30
December	362	9·0	379	54
1918				
January	140	0·0	150	24
February	72	3·0	100	10
March	69	0·0	252	32
April	41	2·5	124	28
Total*	1102	1·9	5789	18

* These percentages have been worked out from the total number of specimens found infested each month.

During the period of maximum infestation the canals are full of irrigation water resulting from the annual rise of the Nile—this would appear to be one factor.

During the months of January and February, according to our observations, a large number of the adult snails die off, and, in fact, the survivors are very difficult to find as they remain inactive at the bottom of the pools. In April and May these canals are periodically flushed with a large addition of stored water, and it is at this time that the maximum breeding of *Planorbis* takes place. For then large floating masses of young *Planorbis* adhering to the weeds collect in stagnant backwaters in the native villages, and, owing to the proximity of the native houses, they stand a very good chance of becoming invaded by miracidia.

The breeding season of *Bullinus*, on the other hand, from observations we were able to make in the sedimentation tanks at Kantara, would appear to be the months of July and August, when the water in most of the smaller canals is at low ebb. At this period also, this snail is most active and appears on the surface of the water, where it is scarcely ever found at other seasons. Owing to the small volume of water in the canals it is legitimate to assume that this is the season wherein the maximum opportunity of being brought into close contact with the miracidia of *S. haematobium* occurs.

The same rule that appertains to bilharzia also appears to hold good for the developmental stages of other trematodes. The statistics in Table V show that the *Bullinus* harbour the greatest number of these also in the month of December. The increase during this month is due to the increase of pigmented cercariae of *Gastrodiscus aegyptius* which is a parasite of the buffalo. According to our experience, this parasite is found mostly at this period of the year, and not during the height of summer, see Table V.

Table V.

Dissections of Bullinus contortus *and* Bullinus dybowski *for presence of other cercariae* (*Cercariae of* Gastrodiscus aegyptius, *and those figured in Plate III, figs.* 5, 6, 7).

Month	Number of snails dissected	Percentage infested as above
1917		
May	51	39
June	27	18
July	140	31
August	57	29
September	93	20
October	No record	No record
November	No record	No record
December	362	75
1918		
January	140	57
February	72	70·8
March	18	70
Total	960	54

In summarizing these results we might state:

(1) That in contradistinction to the observations of Looss we have found throughout the winter months, marked growth and multiplication of laval trematodes—*S. haematobium*, *S. mansoni*, *Gastrodiscus aegyptius*, and others.

(2) That while the month of maximum infectivity for both species of Bilharzial cercariae was December, there was definite evidence to show that *endemically areas are potentially infective throughout the whole year.*

Description of Non-Eyed, Fork-Tailed Cercariae found in Fresh-Water Snails in Egypt.

Only two of these were found and both are obviously not connected in any way with disease in man; one a very small fork-tail cercaria in *Planorbis mareoticus* from the Zoological Gardens, Giza. The other was obtained in 4 per cent. of a batch of *Bullinus dybowski* in the sweet-water canal by the Little Bitter Lake. We have also been able to examine the cercaria of *S. spindale* from India, kindly sent by Lt-Col. Glen Liston, I.M.S.

1. Bilharzia-like cercaria from *Planorbis mareoticus* (Plate III, fig. 5). The measurements of this cercaria were as follows:

Total length	0·296 mm.
Body	0·119 mm. × 0·043 mm.
Tail	0·177 mm.
Fork	0·044 mm.

This form differs materially in having a marked and elongated oral sucker, armed with numerous and prominent papillae. Pigmented eye spots were seen in the specimens collected by Leiper, but were not present in those we examined, as perhaps they were too immature. The ventral sucker is round and comparatively large. In front of it lie two pairs of salivary-mucin cells; the tail is rather broad and provided with short forks. The integument is covered with minute spines. The sporocyst is an oblong oval measuring 0·119 mm. by 0·102 mm. filled with granular germ cells and is easily dissected out from the liver substance (Plate III, fig. 6). According to Leiper this cercaria is probably the larval form of the *Bilharziella* of the duck.

2. Bilharzia-like cercaria from *Bullinus dybowski* (Plate III, fig. 7). The measurements of these cercariae were as follows:

Total length	0·311 mm.
Body	0·119 mm. × 0·031 mm.
Tail	0·192 mm.
Fork with cuticular expansion	0·150 mm.

This can easily be distinguished from the true Bilharzia cercaria by its narrow elongated head, and very markedly forked tail with its cuticular expansions. Each arm of the fork approximates that of the total length of the tail. The oral sucker protrudes slightly and is armed with many minute papillae; there are three pairs of salivary-mucin cells around the ventral sucker, and there is a marked posterior expansion of the oral sucker and a rudimentary pharynx. The body and tail are covered with minute spines.

The sporocysts are elongated finger-like structures (see Plate III, fig. 8), apparently emanating from a common centre. In the outer layer of the cyst are numerous amber coloured granules. Individual cysts vary in size, the largest measure 0·678 mm. by 0·075 mm.

This cercaria appears to us to be identical with *Cercaria gladii* from *Isodora schakoi* of S. Africa described by Cawston and recently figured by Faust (1919). Our measurements are, however, smaller than his.

Both these cercariae differ materially from the fork-tailed cercariae with well-marked pharynx, but without eye spots, sent to us by Lt-Col. Glen Liston, I.M.S., from *Planorbis* in India and which has been found to be the larval stage of *Schistosomum spindale* of the goat (Liston, 1918). These measure about 0·420 mm., total length, and roughly resemble similar organisms figured

by Cawston (1915, 1917) as occurring in *Physopsis africana*, though his figures lack accuracy and detail. The measurements of this Indian cercaria we procured are as follows:

Total length	0·420 mm.
Body	0·162 mm. × 0·047 mm.
Tail	0·260 mm.
Fork	0·092 mm. × ·021 mm.

For the purpose of comparison, and to aid future workers, a short description is given of other cercariae found in *Bullinus*, *Planorbis* and a few other common species of fresh-water snails in Egypt, as figured in Plate III.

Plate III, fig. 9. *Cercaria cellulosa*, a microcotyledonous distome cercaria (spec. inq. Looss, *Rech. sur la faune parasit. de l'Egypte*, p. 227, Pl. XIV, figs. 159, 161). Small, hyaline, very active and found in 10 % of *Cleopatra bulimoides* from Tel-el-Kebir. Looss found them also in *Melanio tuberculata*.

The anterior sucker is provided with a hyaline dart. Opening out into it are two pairs of well-marked cephalic glands. The ventral sucker is round, and is situated immediately posteriorly to the central line of the body; laterally placed to it, are collections of genital cells. The excretory vesicle is apparent in the posterior part of the body. The sporocysts are round, or sacciform (Fig. 10) and are unpigmented.

Plate III, fig. 11. Microcercous distome cercaria found commonly in liver of *Limneae* (mostly *L. caillaudi* and *L. truncatula*) resembles *Cercaria pusilla* spec. inq. of Looss, shows two pairs of cephalic glands with ducts opening into oral sucker, a muscular pharynx and well-marked ventral sucker. Excretory bladder situated at the posterior part of the body, and two excretory canals opening into corresponding vesicles. In the oral sucker is situated a hyaline dart.

Plate IV, fig. 1. Under this heading are included probably amphistome cercaria of three species as described by Looss. They are of a large size, about 0·84 mm. in length, by 0·427 mm. in breadth. The body is oval and almost circular, and in the more mature forms is so darkly pigmented that no details of structure can be made out. They resemble indeed, both in size and movements, minute tadpoles. They are found most commonly in *Bullinus*—about 5 %, but were occasionally seen in *Planorbis boissyi* also, as well as in *Limnaea caillaudi* and *Cleopatra bulimoides*. The pigmentation of the body seems to commence from two eye spots situated in the anterior third of the body and thence to radiate along the excretory and alimentary canals till the whole body is thus pigmented. The anterior sucker is round and smaller than the posterior, which is also circular and muscular, and is situated at the extreme posterior end of the body. The oral sucker leads into a muscular oesophagus, and eventually into a semicircular branched alimentary canal. The excretory system is represented by a series of refractile globules passing in a circular manner from the anterior to the posterior sucker. The tail is elongated and tapering. These cercariae are probably larval stages of *Gastrodiscus aegyptius* (Cobbold) of the horse, of *Gastrothylax gregarius* (Looss) and of *Amphistomum conicum* (Rud.) from the stomach of the buffalo.

Plate IV, fig. 2. Mature redia of Fig. 1; surprisingly small considering the size of the cercariae. They are obtuse oval in shape and hyaline without any protrusion or appendix and measure 0·77 mm. by 0·13 mm. and show large germinal masses in their cavity. At the posterior pointed extremity there are some well-marked germiniferous cells.

Plate IV, fig. 3. This is a monostome cercaria, the *Cercaria pleurolophocerca* of Sonsino (1895) and Looss (1896). We found it occurring in *Melania tuberculata*, both from el Marg and Tel-el-Kebir. Its total length is 1·00 mm. The body is 0·27 mm. by 0·08 mm. The tail is 0·49 mm. in length and is provided with a lateral cuticular expansion. In shape the body is an elongated oval tapering towards the anterior sucker. The cuticle is clothed externally by a number of minute

spicules. The circular oral sucker opens into a short muscular pharynx. No more of the alimentary canal can be made out, and according to Looss, this is absent. The remainder of the body is occupied by eight pairs of large cephalic glands whose numerous ducts open into the mouth cavity. Situated in the posterior sixth is an oval structure with cellular outline and an absence of a muscular rim; this was thought by Sonsino to be the ventral sucker, but Looss pointed out (*op. cit.* p. 209) that it represents the excretory vesicle and that the cercaria is probably the larval form of some monostome. In front of this excretory vesicle is situated a round well-marked collection of genital cells. The tail is long and tapering and in the more mature forms is provided with a lateral cuticular expansion as is shown in the Plate.

The rediae are sausage-shaped striking objects, 1·055 mm. in length and 0·150 mm. broad, and possess a muscular anterior sucker. They are granular in appearance and contain a number of cercariae in all stages.

Plate IV, fig. 4. Distome cercaria from liver of *Cleopatra bulimoides* commonly found at el Marg and at Tel-el-Kebir. This is evidently the *Cercaria distomatosa* of Sonsino (1895). Rediae of this species are large and pigmented and form very prominent objects. The whole body appears granular, so occupied is it with cystogenous cells that it is only with difficulty that the finer points of internal structure can be made out. The ventral sucker (acetabulum) is muscular and prominent; the oral sucker is pitted round its margin with numerous small orifices, said by Looss to be the openings of many small ducts. The pharynx is continued into a muscular oesophageal bulb. Posterior to this, the two blind sacs of the intestinal canal branch off and run into the posterior end of the body. The excretory system is represented by two fine excretory ducts, which run from the ventral sucker and open into a vesicle at the posterior end of the body. The tail is blunt-ended and does not move independently when the animal is alive. The extremity of the tail is rounded and is split for a short distance at its termination. The excretory canals open bilaterally on the anterior portion of the tail.

Plate IV, fig. 5. Very active amphistome cercaria commonly found in *Bullinus*, *Planorbis* and once in *Physa*; apparently not described by Looss. In the former snail from el Marg as well as from Tel-el-Kebir 31 % were found to be naturally infested. The body is full of cystogenous cells. The oral sucker is round with an oval termination. There is a large muscular pharynx which imparts a lobulated appearance to the anterior end of the body. This is bordered on one side by a string of glands, the canals of which run forwards and open laterally into the mouth. There is a straight oesophagus which branches into two ill-defined alimentary caeca anterior to the ventral sucker. The branches of the alimentary canal run far forward, and also for a short distance towards the posterior end of the body. The ventral sucker is large, prominent and muscular; on its anterior margin there opens a small duct derived from a collection of cells (probably genital) situated at its posterior border. The tail which is equal in length to the body, is long and tapering, and contains the efferent excretory canal. This cercaria is often found encysted in the liver of *Bullinus* and *Planorbis*, especially in the winter months (Plate IV, fig. 6).

The redia and cercariae appear to resemble those described by Gilchrist who has worked out their life history in S. Africa and finds them to be the larval stages of *Distomum luteum* in the intestine of the frog, but our measurements are considerably larger than his.

This is possibly also the *Cercaria pigmentata* described by Sonsino.

Plate IV, fig. 7. The redia is a very striking object. The infested livers in which they are found are of a bright orange colour, and the organ appears to be packed with them. Directly the shell of such an infested snail in incised, these prominent objects can be seen floating out of the breach. The body wall of the redia is studded with bright orange pigment granules. The length is about 1·43 mm. There is a large anterior muscular sucker, and near the posterior end there is generally a lateral protrusion or appendix. The interior of the redial sac is filled with young and active cercariae. (Corresponds with that of *Distomum luteum* of Gilchrist.)

For compiling this section the works of Cort on N. American Cercariae and of Faust on those of S. Africa have been consulted.

V. CONCERNING INVESTIGATIONS OF THE SWEET-WATER CANAL TO WHICH INFECTION AMONGST AUSTRALIAN AND IMPERIAL TROOPS WAS TRACED, AND THE CONCLUSIONS BASED UPON THEM.

The following incidents form a very remarkable and practical corroboration of Leiper's work.

In October and November, 1916, several cases of bilharziasis were admitted to No. 14 Australian General Hospital. In these, examination not only revealed lateral spined ova in the faeces, but also terminal spined ova in the urine. It was therefore suggested as possible that most of the cases from Tel-el-Kebir in Lawton's series, to which reference will be made later, were mixed infestations. As no *Bullinus* had been found, when inspection following this outbreak was made, we re-investigated the Rifle Range Canal at Tel-el-Kebir, and succeeded in demonstrating *Bullinus* infested with *Schistosomum haematobium*, as well as *Planorbis* infested with *Schistosomum mansoni*.

In addition it was found that the fellaheen in the adjacent fields were passing many lateral spined ova in their faeces, terminal spined ova were found in the urine of two out of three cases examined. With the cercariae from infested *Planorbis* gathered at Tel-el-Kebir, we succeeded in infesting monkeys, and thus proved conclusively the infectivity of the canals in this area.

A careful analysis of the histories of patients admitted to Hospital suffering from bilharziasis, soon led to the conviction that other areas than Tel-el-Kebir were responsible for infestations amongst Australian Light Horse. Pools at Serapeum and Abou Soueir were suggested as sources of infestation. Further, the cases from Serapeum were all simple infestations with *Schistosomum haematobium*, while those from Abou Soueir were infested with both species. It was possible to predict therefore, on the basis of Leiper's works, the presence in any particular area of *Bullinus* or *Planorbis*, or of both. In every case subsequent investigations proved the truth of this prediction.

Table VI.

Canal zone	Infective bathing areas	Troops affected	Total Number of cases	Type of infestation			Species of snail found in pool and locality	
				Schist. mansoni	*Schist. haematobium*	Double infestations	*Planorbis*	*Bullinus*
Zag-a-zig to Ismaila	Tel-el-Kebir	Australian Light Horse	49	6	10	33	present	present
Zag-a-zig to Ismailia	Abou Soueir	,,	6	...	4	2	present	present
Kantara to Suez	Serapeum	,,	14	...	...	14	absent	present
Upper Egypt	Deirut	,,	11	...	11	...	absent	present
Upper Egypt	Fayoum	Imperial Mounted Yeomanry	6	...	6	...	absent	present

NOTE. The actual pool at Deirut in Upper Egypt was not investigated, but reference to the literature shows that snails of the *Bullinus* species are found there, but not those of *Planorbis*. McCallan (1915) was able to show a 25 % infection with *S. haematobium* in the country district around Deirut.

An analysis of Table VI (attached) affords a remarkable proof of Leiper's work in demonstrating the specific relation of the two types of *Schistosomidae* and their intermediary hosts.

A description of the following bathing sites well illustrates the variable modes in which infection may occur.

1. At Tel-el-Kebir the troops bathed in a pool formed by the expansion of a narrow sweet-water canal (Pl. V, fig. 2).

2. At Abou Soueir they became infested by swimming in a much wider and deeper sweet-water canal.

3. At Serapeum they became infested by washing in a shower bath under an overflow pipe which conveyed water from the adjacent sweet-water canal.

(1) *Pool at Tel-el-Kebir* (Pl. V, fig. 2). Close to the Rifle Range at Tel-el-Kebir and running on the edge of the cultivated land between it and the desert, is a fresh-water irrigation canal not more than eight feet across at its widest, nor more than three feet deep at any part. There is a clump of shady fir trees near the Rifle Range Road, and at this point is a small sluice gate, beyond which the canal widens out into the pool in question. It is about 60 feet long and 30 feet wide by four feet deep. Beyond this the canal runs on again, lined at this point with reeds and rushes. After a morning's musketry, it was a common practice for the troops to have lunch under these trees, and many bathed in the pool, while others drank the canal water. In this way the disease was contracted.

On investigating this bathing pool and the canal, we found many snails of the species *Planorbis boissyi* floating down stream, attached to débris or joined together in masses to form rafts. It was the predominant snail of the mulluscan fauna of this area. Examination of the submerged rushes and the stems of the water-grasses brought to light *Bullinus* snails in quite large numbers. A collection of snails of both kinds was made and on dissection, the cercariae, in one case of *S. haematobium*, and in the other of *S. mansoni*, were easily demonstrated. Thus, out of 513 snails of the species *Planorbis boissyi* five, or 1 per cent. were found to be infested, while of 190 specimens of *Bullinus* two, or 1 per cent. (approximately) were found to be in a similar condition.

The water level of this pool was found to vary on different days, as did also the molluscan fauna it contained. From this it was evident that its potentiality as a source of infection could vary within wide limits in quite a short space of time. In some such manner we may account for the fact that while the great majority of cases became infested with both species, a few acquired only *S. mansoni* and fewer still *S. haematobium* alone.

A point of great interest was the rapidity and comparative ease with which many of these molluscs, particularly *Planorbis boissyi*, travelled over quite wide areas by adhering to floating débris. It seems probable from this that snails originally infested from human excreta in the densely populated regions like Zag-a-zig, and perhaps even the environs of Cairo itself, through which the

canal passes, may be widely distributed over the less densely populated agricultural areas of the Delta. The flooding of practically the whole of the Delta, which takes place annually, is no doubt a very potent factor in the spread of the disease.

(2) *The Canals at Abou Soueir.* During the summer of 1916, a troop of Light Horse engaged in patrolling this sector was camped on the banks of the canal. In the hot evenings the men were in the habit of swimming in this canal, which is the second largest in the vicinity. It is deep enough for swimming and is from 40 to 60 feet in width, and its banks are lined with reeds and rushes. Investigation showed the presence of snails of the following species: *Bullinus contortus, Bullinus dybowski* and *Planorbis boissyi.*

As in the Tel-el-Kebir group, so in this series also, two of the cases were found clinically to be double infestations, while four were infested with *S. haematobium* only.

(3) *Serapeum* (Deversoir). Troopers of the Australian Light Horse stationed here were in the habit of bathing in the Suez Canal, which at this point enters the northern end of the Little Bitter Lake. After their swim, it was customary for a number of them to cross a belt of marshy land, about 50 yards wide, for a final shower in the fresh water of an over-flow pipe from the sweet-water canal. For this purpose they stood in a small pool about 3 feet in diameter, by 2 feet deep, formed by the falling water. In this way most of them contracted bilharziasis.

Subsequent investigation of the canal from which the pipe was leading, of the pool itself, and of the adjacent canals, revealed the presence of *Bullinus*, and of *Physa*, but only three specimens of *Planorbis boissyi* were found after an exhaustive search. Amongst the cases of bilharziasis from this zone, no infestation with *S. mansoni* was detected, though the faeces were most carefully examined in every case.

(4) *The Fayoum.* In 1917 a very considerable number of urinary cases (*S. haematobium*) occurred amongst the Yeomanry detachments; the infestation being originally contracted in 1916. Eleven of these cases have come directly under our personal observation. Terminal spined ova were found in their urine, but lateral spined ova were never demonstrated in their faeces.

In this district we found that *Bullinus contortus* was widely distributed, but *Planorbis boissyi* was never observed, after exhaustive search on two sepa rate visits to the ponds, ditches and canals of this oasis.

The Village at el Marg. Before concluding this section the following observations made at el Marg (Delta district) situated 12 miles north-east of Cairo will be included.

Although we have no statistics of white troops billeted in this locality, the relationship between the molluscan fauna, and the types of bilharziasis occurring in that area, has been noted.

In the birkets and canals large numbers of *Planorbis boissyi* are always to be found, and greatly out-number any other species of fresh-water snail. *Bullinus*, on the other hand, is not so common. On reference to Table IV it will be seen that from over 5000 dissections of these snails, 18 per cent. were infested with *S. mansoni*. On the other hand, in over 1000 dissections of *Bullinus*, only 1·9 per cent. were found to be infested with *S. haematobium*.

In el Marg at any rate it would seem that the occurrence of rectal bilharziasis in man ought to be much more common than that of urinary form. As a matter of fact both rectal and vesical forms are exceedingly frequent amongst the native population, though from its situation the former bilharziasis (*S. mansoni*) requires more painstaking observation for its detection.

A glance at the map of Egypt will show that the bathing sites where troops acquired infection are widely spread over various stretches of fresh water, including those of both Lower and Upper Egypt.

Thus, Tel-el-Kebir and Abou Soueir are on the Zag-a-zig to Ismalia canal; Serapeum is situated on the sweet-water canal between Kantara and Suez; Deirut and the Fayoum are both in Upper Egypt.

They therefore serve, in a general way, as excellent indications of the potentialities of these particular spots.

CONCLUSION.

As a result of this study of the clinical types of bilharziasis originating in a given locality, and from subsequent investigation of the molluscan fauna of that area, we are presented with a remarkable corroboration of Leiper's work. The specificity of these two species of snails for their respective parasites has, in this fashion, been repeatedly proved.

It is surely very satisfactory to be able to substantiate that the distribution of the disease in man corroborates in such a remarkable way the experimental facts observed in the laboratory.

Wherever the Planorbis *is the predominant species there will rectal bilharziasis be found, and wherever* Bullinus *abounds there the urinary form of the disease will be the most prevalent.*

The statistics given in Table VI show how heavily parts of the Delta district are infested with *S. mansoni*. From these statistical studies, from clinical observation, and from other data, we are firmly convinced that intestinal bilharziasis (*S. mansoni*) is much more prevalent amongst the Egyptian population of Lower Egypt than is at present recognized.

Intestinal bilharziasis is more difficult to diagnose clinically for the following reasons:

(1) Intestinal symptoms in man are frequently latent, and when present do not, in a tropical country, attract so much attention as do urinary ones.

(2) Many more ova are produced by *S. haematobium* than by *S. mansoni*, and therefore the former are much more easily found under the microscope in routine examination of the discharges.

Distribution of fresh-water snails in localities visited in Egypt.

El Marg near Cairo — *Planorbis boissyi* (common), *Bullinus contortus, dybowski, innesi, forskali, Physa subopaca* (scarce), *Vivipara unicolor, Bythinia senaarica, Cleopatra bulimoides* (very common), *Melania tuberculata, Limnaea caillaudi* (scarce).

Zag-a-zig to Ismailia — *Bullinus contortus* and *dybowski, Planorbis boissyi, Limnaea caillaudi, Cleopatra bulimoides, Physa subopaca, Melania tuberculata, Planorbis mareoticus.*

Kantara Tanks — *Bullinus dybowski, Physa subopaca.*

Southern Canal Zone — *Bullinus dybowski* (much the commonest species), *Bullinus contortus* (rarer). Very few *Planorbis boissyi*. Amongst 1000 *Bullinus* there were only three *Planorbis*.

Cairo

1. Head Storage Reservoirs. Abbassia (unfiltered water supply) water collected directly from the Nile.
   - *Bullinus contortus* (very common).
   - *Bullinus dybowski.*
   - *Limnaea caillaudi.*
   - *Melania tuberculata.*
   - *Planorbis boissyi.*
2. Snails from unfiltered water-pipes.
   - *Cleopatra bulimoides.*
   - *Melania tuberculata.*
3. Public Ponds.
   - *Limnaea caillaudi.*
4. Snails from receding Nile at Agu Baba in mud.
   - *Vivipara unicolor.*
   - *Cleopatra bulimoides.*
   - *Bythinia senaarica.*
   - *Melania tuberculata.*

VI. THE INCIDENCE AND ORIGIN OF BILHARZIAL INFECTION AMONGST THE CAIRENES.

As Australia is faced with the potential danger of outbreaks of bilharziasis, the following observations on the manner in which the natives of Cairo are infested may be of importance.

Looss, in an investigation of the percentage of infested children in Cairo, found 33 per cent. of the boys at a certain school to be infested with *S. haematobium*. In 1908, Mrs Elgood showed that 27·5 per cent. of the girls attending a school in Cairo were similarly infested.

The supporters of the "Looss hypothesis" maintained, that as the children had never been out of Cairo, and as there was a common filtered water supply to the white and native population of Cairo alike, direct miracidial infestation was the only explanation that would account for the distribution of bilharziasis amongst them.

In criticizing the supporters of the "Looss theory," Leiper pointed out the presence of a second water system carrying unfiltered water from the Nile, and used ordinarily for supplying public and private gardens. This water,

on account of its cheapness, was being largely used by the natives for domestic, drinking, and other purposes.

At the request of Dr Todd, the Director of the Public Health Laboratories, we inspected the storage reservoirs of this crude unfiltered water supply derived directly from the Nile.

In the two main storage tanks *Bullinus* were found on the green water-weeds growing below the surface. In a small adjacent tank, 15 feet in diameter, 250 *Bullinus* were collected in a few minutes from the inlet pipe that comes from the Nile.

In the old disused filter beds near those at present in use, many thousands of shells of *Bullinus*, and some of *Planorbis boissyi*, were found.

These findings suffice to explain the incidence of bilharziasis amongst the native children of Cairo who have not been outside the City itself, and prove the danger of this unfiltered water supply to the whole community. The molluscan fauna in the ponds and fountains of public gardens of Cairo are also derived from this water supply. We have frequently noted that either the spawn, the young, or the adult snails themselves can withstand the water-pressure in the piping. Thus at el Marg, infested snails may be obtained in quantity in the railway tanks which derive their water supply from adjacent canals.

While the infectivity of the Nile water has never been demonstrated, our observations support those of Leiper on the subject. The presence of snails in the pipe conveying water from the Nile, and the large number of shells left in the mud on the banks when the water recedes, prove the wide distribution of fresh-water snails in that river. Felucca men (boatmen) state that snails are to be found adherent to their boats if they have been anchored for any length of time during the summer months. Several years ago an outbreak of bilharziasis occurred amongst soldiers who had bathed in a specially constructed floating wooden bath, in the Nile at Kasr-el-Nil bridge. Here the conditions for the concentrated action of cercariae were ideal, for snails adhering to the side of the framework nearest the current would create a zone of infectivity in the water immediately beyond.

All along the Nile banks deposits of human faeces may be observed, similarly urinary contamination occurs, owing to the un-hygienic habits of the natives.

Along the banks where the reeds and grasses cause the water to become stagnant, snails may be observed, and it is here that the miracidia from infected excreta have every chance of finding their intermediary hosts. Native water-carriers fill goat skin bags with this water, and sell it to the native population at a very cheap rate. It is used by them for drinking and domestic purposes. This practice may constitute another very real danger to the community.

VII. SUGGESTIONS FOR PROPHYLAXIS.

Prophylactic measures necessarily resolve themselves into two lines of action.

1. Destruction of ova in the excreta prior to contamination of the water.
2. Destruction of the molluscan intermediary hosts of the Schistosomes and the purification of contaminated water supplies.

(1) *Destruction of ova in excreta prior to contamination of the water.* In any highly civilized community an efficient system of sanitation would markedly lessen, if not entirely eradicate, the disease.

In Egypt, however, the Arab inhabitants are far from being highly civilized, and their ideas of sanitation are rudimentary in the extreme, if not entirely lacking. The water in the canals is being constantly contaminated with urine and faeces, and in consequence miracidia are abundant. Especially is this the case in the environs of large cities such as Cairo, Zag-a-zig, etc. With the means at present at the disposal of the Government the problem of educating the fellaheen on sanitary lines is beyond solution; it must be many years, one is tempted to say, centuries, before prophylaxis based on a system of rational sanitation will be practicable.

Introduction of bilharziasis into countries in which it is at present not endemic.

One of the chief topics of medical interest at the present moment is to prevent the introduction of bilharziasis into countries where it is not at present endemic, but where the fresh-water snail fauna may be capable of acting as intermediaries and the climatic conditions are suitable.

Something of this sort seems to have happened in the analogous case of the liver fluke (*D. hepaticum*) when introduced into Australia together with its vertebrate host—the sheep.

The ideal method to prevent the introduction of this disease would be to confine carriers of Schistosome ova to sequestrated regions where their dejecta would be incapable of reaching any water supply in which the suitable fresh-water mollusca could live.

One need hardly point out that the systematic microscopic examination of the urine and faeces of all persons returning from an infected country such as Egypt, to an uninfected one, such as Australia, is almost an insuperable task; and Australia is not alone in facing the problem to-day, for the same condition applies even more strongly to India, where apparently the agricultural and climatic conditions are favourable and the snail fauna similar to that of Egypt but where up to the present transmission experiments with the local *Planorbis*[1] have failed.

[1] Private communication from Prof. Annandale and Dr Kemp.

As we suggested in 1916, the ideal procedure would be to confine those cases known to be infested with bilharzia that have returned to Australia to sewered areas, at all events till they have learned to appreciate their potential danger to any community. These suggestions, we understand, have been carried out.

These measures alone might appear sufficient to prevent the disease ever becoming endemic in Australia, but such is really not the case. We have met with fully 20 cases styled "Latent Bilharziasis." These patients have either no symptoms at all, or their symptoms have been so slight as to be entirely disregarded. Nevertheless, the examination of their excreta has revealed the presence of lateral or terminal spined ova. It is in infestations with *S. mansoni* that latency is most frequently seen. Most of these "latent" cases were diagnosed in a routine examination of a large number of sera by means of the specific complement deviation test for this disease. They have been more fully dealt with in previous publications. (*Journ. Roy. Army Med. Corps*, IV. and VI. 1919.)

From the above it will be seen that the existence of the disease in people who are unaware of, or neglect the signs of, its presence, considerably complicates the problem of the prevention of the spread of this disease into a community. It is the carrier problem over again!

(2) *The destruction of intermediary hosts.* The destruction of the molluscan fauna of a country, such as Egypt, would lead to the disappearance of bilharziasis in man and of a number of "trematode" diseases affecting animals.

In a country with an intensive system of irrigation, such for instance as Egypt, the molluscan fauna becomes a universal and persistent scourge. Many measures have been proposed for the eradication of the intermediate snail hosts.

Dr C. Todd, the Director of Public Health, informs us that several years ago the possibility of reducing the molluscan fauna by means of ducks and wild fowl was carefully considered, but the idea was rejected as impracticable for Egypt.

More recently, Major T. Cherry has advocated this method as applicable to Australia. While it is possible that the presence of ducks and other wild fowl might materially help to diminish the number of molluscs in localized water areas, it is far from being the solution of the problem of Egyptian bilharziasis, and at most can only be regarded as a possible accessory measure.

In 1915, Leiper, in his "suggestions for the eradication of the disease in Egypt," proposed that the annual rotations in the supply of water enforced by the Government from April to August in Lower Egypt, should be utilized to destroy the molluscan fauna.

During the periodical stoppages of water supply for 15 days, the canals become dry except for small puddles. By the systematic use of destructive chemical reagents, Leiper suggested that the number of contained molluscs could be considerably reduced. It may be that methods of this sort, if they could be attempted, would be possible for the smaller, but not for the large canals. There are obviously many grave objections to this scheme, as the whole

agriculture of this rainless country is so dependent upon the life-giving stream emanating from them that the water supply cannot be interrupted for any length of time.

Obviously some other method must be devised more in keeping with the agricultural needs of the country.

THE SPREAD OF THE DISEASE IN EGYPT.

Snails of the two genera *Planorbis* and *Bullinus* differ somewhat in their habits.

As a general rule, *Planorbis* is a surface-feeding snail and favours either slowly moving or stagnant and muddy waterways. It feeds on any vegetable material, but for the most part on the rushes and grasses which grow at the margins of the water. Little difficulty is generally experienced in collecting specimens as they occur in masses floating on the surface.

Bullinus, on the other hand, is generally found attached to stones, posts, etc. at some considerable depth below the surface. It is more usually found in clear water such as in large tanks and in more swiftly flowing waterways than is the former. It feeds upon various kinds of aquatic plants which choke these channels in the summer season and may then be found in clusters attached to the under surface of the floating leaves. The main breeding season of *Planorbis* in Egypt would appear to be the spring months, March and April, while *Bullinus* deposits its ova at a later date.

In the case of *Planorbis* the egg-masses are generally deposited upon the shell of another individual of the same species; while *Bullinis* deposits its ova upon stones at the bottom of the canal. The young snails, according to our observations, take about three months to mature.

From our observations the fact emerges that the highest rate of infestation of fresh-water snails occurs round certain centres in the Delta, such as Cairo, Zag-a-zig and Ismailia. These three towns in particular are situated on the main sources of water supply for the Delta and Canal zone, and from them a maximal faecal and urinary contamination of water takes place. Now it has been shown that the range of action of the miracidium is a comparatively limited one.

In the less sparsely inhabited stretches of the fresh-water canal, between these densely populated centres, it is very improbable that the local snails would become infested with miracidia; or if they did, that the cercariae thus produced would be numerous enough to run any chances of encountering a suitable host during their short lifetime.

During the spring months of the year the sluice-gates are open and the Nile water is coursing through the canals of Lower Egypt bearing with it large masses of fresh-water weed and vegetation. and it is to this that most of the infested snails can be found to be clinging. Therefore it would seem than an annual migration of snails to the most distant parts takes place by these means and that this migration corresponds with the breeding season.

From these observations we are led to believe that these towns act as foci in the distribution of bilharziasis to other parts of Egypt.

It would seem possible to greatly minimize the spread of the disease by attaching some kind of mechanical device, such as a wire gauze filtering apparatus, to the lock gates so as to intercept the masses of floating weeds with the contained snails which could then be thrown on to the banks and destroyed. At any rate it would capture many mature and infested snails and thus materially lessen the number capable of carrying the infection.

There is one point as regards the prophylaxis which requires explanation and that is why *S. haematobium* infestations occur so commonly in Egypt, while at the same time it is so difficult to find *Bullinus* infested in any numbers. During the course of routine snail dissections this has been a constant source of speculation.

The highest percentage of infested *Bullinus* we have recorded for one month is 9 per cent.— more commonly it is 1 per cent., or even less; in the same locality the average infestation rate for *Planorbis* with cercariae of *S. mansoni* was 18 per cent., and occasionally reached as high as 54 per cent.

Taking all the year round the *mansoni* infestations in snails were nine times commoner than *haematobium* and add to this the fact that *Bullinus* is much the rarer snail of the two. There is therefore enough scope for further enquiry into this most puzzling aspect of this subject. It may be that there are other more efficient intermediary hosts of *S. haematobium*.

It is important to prevent the entry of infested snails into storage tanks. This can easily be done by guarding the intake with a covering of fine-meshed wire, but it is insufficient to prevent the entry of the minute newly hatched mollusc.

Such tanks utilized in the canal zone during the Egyptian campaign soon became infested with multitudes of *Bullinus* which passed through the gauze-covered intake from the sweet-water canal and which found in this situation a suitable habitat.

Observations showed that snails do not become infested with cercariae till they have reached maturity, that is about the third month, so that there is no danger to be apprehended from such a circumstance, provided that the tanks are frequently cleansed and that necessary measures are taken to guard against any faecal or urinary contamination. Of course their presence in drinking water constitutes a potential danger and is therefore undesirable.

It is already known that in Western Australia certain cases of vesical bilharziasis occurred after the South African war in persons who had never travelled outside Australia. It follows therefore that some Australian molluscs must be capable of acting as intermediate hosts for *S. haematobium* at least.

Under laboratory conditions, the determination of the infectivity of Australian molluscs for both *S. haematobium* and *S. mansoni*, the identification of those species capable of artificial infestation, and the investigation of their life-history, habits, and geographical distribution, are all problems which

urgently require solution. On this knowledge must be based any rational prophylactic measures for the eradication of these potential molluscan intermediaries in Australia and in India as well.

PURIFICATION OF WATER SUPPLIES.

In a country infested with endemic bilharziasis, the presence in a water supply of infested *Planorbis* or *Bullinus*, or even uninfested snails of these species, is sufficient evidence on which to condemn that supply, and to put it out of bounds for troops for drinking, or bathing purposes.

Even when the molluscs have been removed, the water may still harbour cercariae for 36–48 hours. Storage over this period is the most satisfactory method of ensuring its safety. Boiling, naturally, is efficient but impracticable. Certain chemical agents are advocated but they appear less lethal for cercariae than for bacteria.

The commonest bactericide utilized in the Army is bleaching powder—1 part of available chlorine per 1,000,000 parts of water, being considered efficient. In the small book published by the War Office (Memorandum on some Medical Diseases in the Mediterranean War Area), it is stated that one part per 1,000,000 of available chlorine is efficient in killing cercariae. In numerous experiments we have proved the fallaciousness of this statement. Thus, after 2½ hours' immersion in water containing 4 parts per 1,000,000 available chlorine, we have found cercariae alive and very motile. The cercariae were obtained from *Planorbis boissyi*, and the bleaching powder used contained 28 per cent. available chlorine.

One part per million of available chlorine is the maximum that can be added without making the water quite unpalatable. Therefore, unless much greater quantities be added, and some method of dechlorinating afterwards employed, this method of purification of water infested with bilharzial cercariae must prove both unsafe, and unsatisfactory.

PERSONAL PROPHYLAXIS.

This applies especially to Europeans visiting those parts of Egypt in which bilharziasis is endemic.

In the case of troops it is advisable to explain to the men, by means of lectures and demonstrations, the dangers they incur by not obeying the rules drawn up for their guidance. After an ocular demonstration of cercariae in the living *Planorbis*, for instance, they will be much more impressed than by any number of printed notices or orders.

To the sportsman a knowledge of this disease is of the greatest importance. It has been suggested that there is little danger of contracting the disease in the winter season when the snipe and duck most abound, as owing to the low temperatures the snails will not be infested.

From actual observations this does not appear to be the case. The snails once infested remain so for life and attain the maximum degree of infectivity

during the late autumn months. During the early part of the year the rate of infestation appears to be a low one; this circumstance is possibly due to the fact that the larger and more adult molluscs (which are those most generally infested) have been swept away by the winter rains.

There is therefore very considerable risk in contracting bilharziasis by snipe shooting, especially in flooded fields and marshes in the *vicinity of native villages*.

It is necessary to remember that the fresh-water snails are not found in brackish water, nor are they present in swamps which dry up in the summer months, as they cannot survive prolonged desiccation. There are also many large tracts of shooting ground in Egypt, the Fayoum, in which, for some reason or other, neither *Bullinus* nor *Planorbis* occurs.

The sportsman visiting an area infested with bilharziasis should be provided with rubber waders which should reach well above the umbilicus; but it is not sufficient to trust to high boots or thick clothing alone, as the cercariae can easily penetrate their interstices.

There is considerable danger in fishing also; several instances have occurred in Egypt in which soldiers have contracted the infection while thus engaged, apparently from handling newly caught fish in the sweet-water canal. Those addicted to fishing should be warned, on this account, to wear rubber gloves.

Similar precautions must be taken by those whose duties consist in procuring water from canals for bathing purposes. Cases of infection have occurred in men detailed to supply water for baths, horse-troughs, etc.

Finally it must be remembered that there is considerable danger even in riding through infested water, as for instance, in watering horses. Mounted troops in Egypt were apt to be offenders in this respect and constantly had to be warned about it.

Men who conscientiously adopted every other precaution against bilharziasis have been observed "swimming" their horses in a very dangerous locality.

ACKNOWLEDGMENTS.

In completing this Report we should like to place on record our appreciation of the manner in which the investigation has been facilitated and encouraged.

The Laboratory Staff of No. 14 Australian General Hospital—the late Staff-Sergeant C. F. Sullivan, B.Sc., Corporal R. C. Stephens, and Private E. Panelli—have rendered us invaluable assistance and have frequently worked overtime in order to facilitate this work.

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DESCRIPTION OF PLATES III—V.

BILHARZIA CERCARIAE AND ALLIED SPECIES FOUND IN EGYPT.

PLATE III.

Fig. 1. Lateral spined ovum of *S. mansoni*: measurement 0·16 mm. × 0·06 mm.

Fig. 2. Terminal spined ovum of *S. haematobium*: measurement 0·15 mm. × 0·056 mm.

Fig. 3. Fork-tailed Cercaria of *S. mansoni*, from liver of *Planorbis boissyi*, showing anterior and ventral suckers, mouth armature, six pairs of poison cells, unpaired glandular structure situated in front of ventral sucker, and posterior pairs of flame cells.

Compiled from average measurements of 40 specimens.

Measurements:

Total length from anterior sucker to fork 0·374 mm.
Body 0·161 mm. × 0·060 mm.
Antero-posterior measurement of anterior sucker 0·053 mm.
Posterior sucker situated 0·044 mm. from posterior border of body.
Length of tail to fork 0·213 mm. Length of fork 0·066 mm.

Fig. 3 *a*. Side view of Head of Cercaria of *S. mansoni*, showing appearance of ventral sucker.

Fig. 4. Fork tailed Cercaria of *S. haematobium* from liver of *Bullinus dybowski*, showing internal structure as in 3, with three pairs of poison cells.

Compiled from measurements of 35 specimens.

Total length 0·398 mm. Body 0·190 mm. × 0·064 mm. Antero-posterior measurement of anterior sucker 0·073 mm.
Posterior sucker 0·049 mm. from posterior border. Length of tail 0·208 mm. Length of fork 0·081 mm.

Fig. 4 *a*. Side view of Head of Cercaria of *S. haematobium* showing oval shape of anterior sucker, and protrusion of ventral sucker.

Fig. 5. Unpigmented fork tailed Cercaria without eye spots, from liver of *Planorbis mareoticus*, from the Zoological Gardens, Cairo. (*Bilharziella*)?

Compiled from measurements of 6 specimens.

Total length 0·296 mm. Body 0·119 mm. × 0·043 mm.
Tail 0·117 mm. Fork 0·044 mm.

Fig. 6. Sporocyst of 5 from *Planorbis mareoticus*, 0·119 mm. × 102 mm.

Fig. 7. Fork-tailed Cercaria from liver of *Bullinus dybowski* taken in sweet-water canal.

Total length 0·311 mm. Body 0·119 mm. × 0·031 mm. Tail 0·192 mm.
Fork 0·150 mm. 4 % of snails taken on 2. viii. 17 infected.

Fig. 8. Sporocyst of 7 showing yellow pigmented granules in cyst wall.

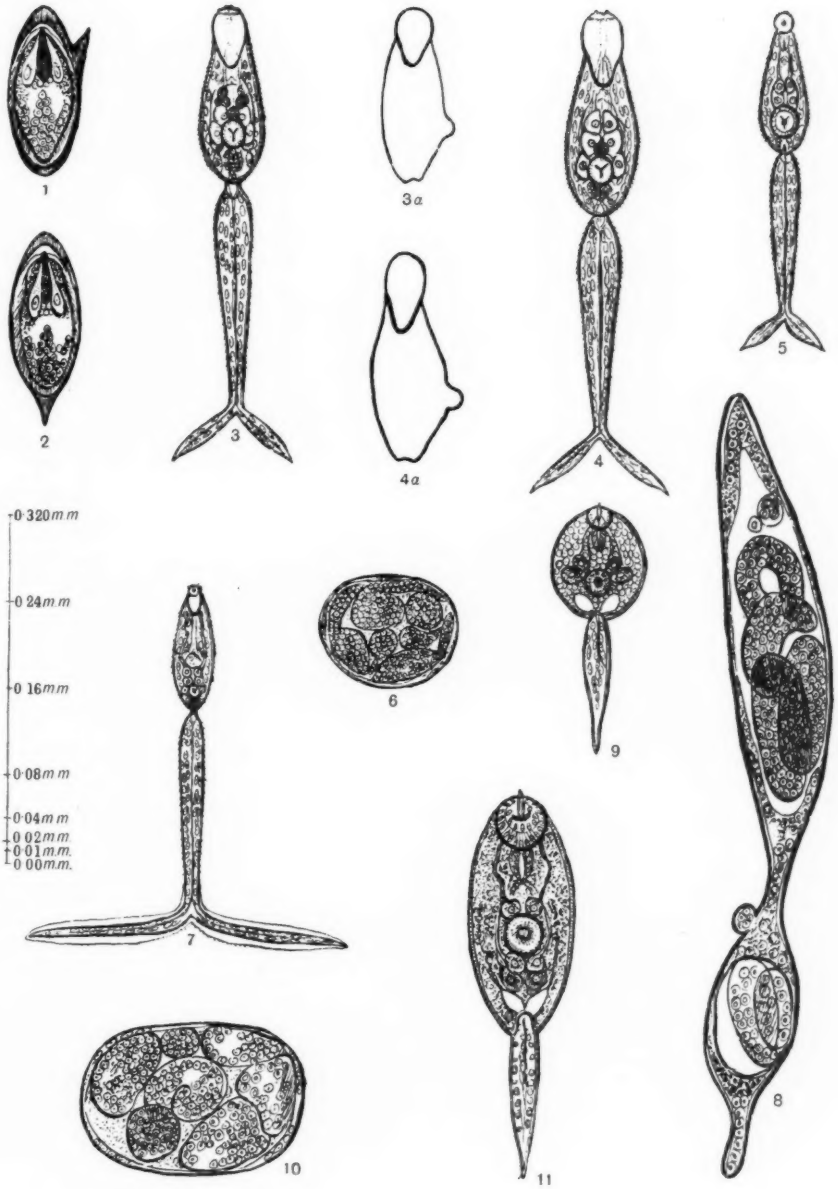
Average length of mature sporocyst 0·678 mm. × 0·075 mm.

Fig. 9. Cercaria found in 10 % of livers of *Cleopatra bulimoides* described and figured by Looss, as *Cercaria cellulosa*, sp. inq. (*Rech. sur la faune parasit. de l'Egypte*, text page 227, Plate XIV, figs. 159–161).

Average total length 0·192 mm. Body 0·102 mm. × 0·06 mm.
Tail 0·09 mm.

Fig. 10. Sporocyst of 9; Average measurement 0·210 mm. × 0·13 mm.

Fig. 11. Cercaria from liver of *Limnaea caillaudi* from the Zoological Gardens, Cairo. (12 % infected.) Total length 0·310 mm. Body 0·195 mm × 0·086 mm. Tail 0·114 mm. Resembles in structure *Cercaria pusilla* described by Looss.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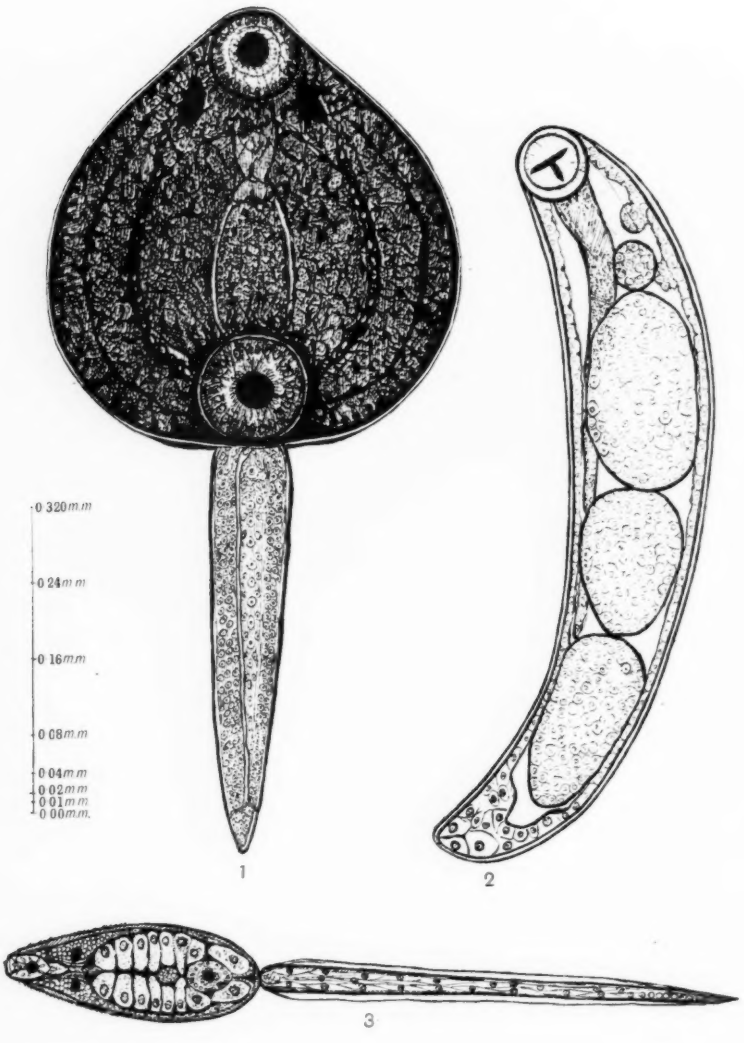

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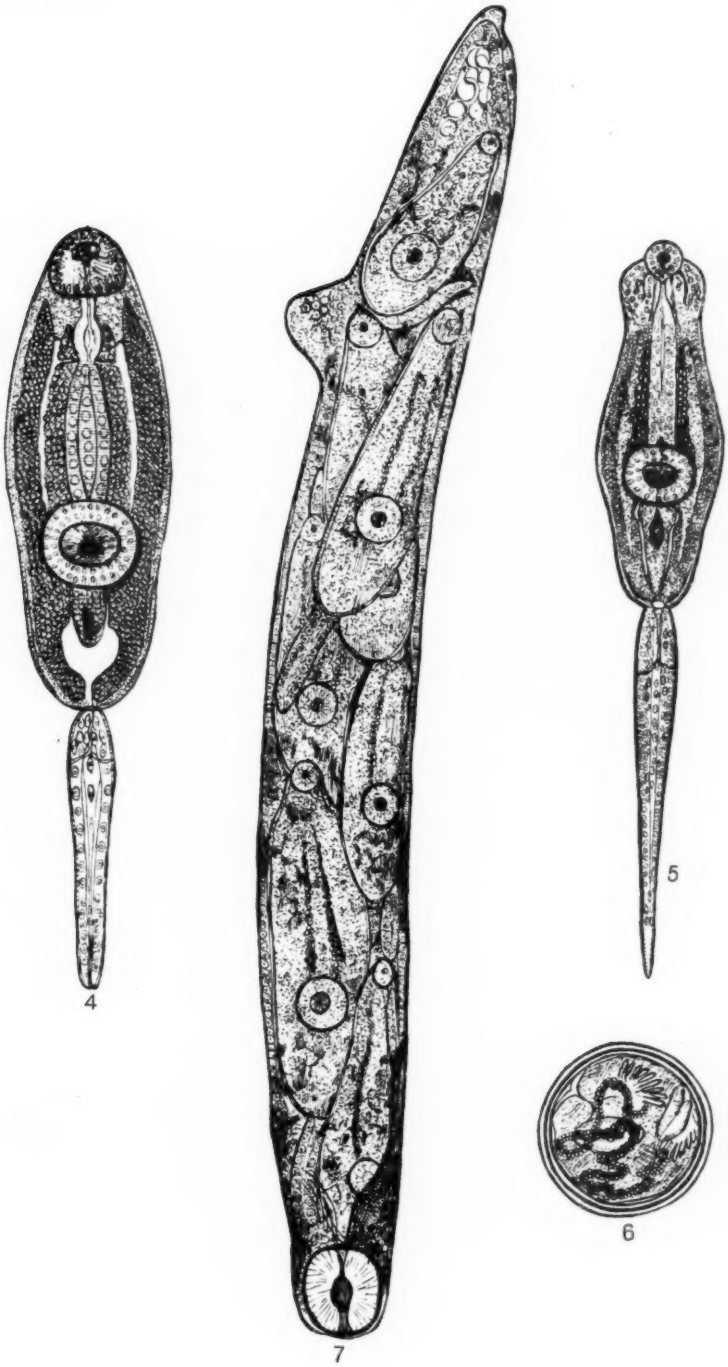
PLATE IV



P. H. Manson-Bahr del.

Fig. 1

Transverse section of liver of *Planorbis boissyi* containing large numbers of sporocysts and cercariae of *S. mansoni*, showing the proliferation of the "germ balls" from the sporocyst wall and the development of the cercariae from them. The small amount of normal glandular tissue remaining is well seen. × ca. 100 diameters.

AL = branch of alimentary canal.
GL = normal glandular tissue.

P. H. Manson-Bahr del.

Fig. 2

RIFLE RANGE CANAL AT TEL-EL-KEBIR

where troops became infested with *S. mansoni* and *S. hæmatobium*

PLATE IV.

Fig. 1. Large Pigmented Cercaria of *Gastrodiscus aegyptius* (Cobbold), *Gastrothylax gregarius* (Looss), or of *Amphistomum conicum* (Rud). The former is a parasite of the horse, the latter two of the buffalo. Looss followed the development of the former, in *Cleopatra bulimoides*, and of the latter two, in his *Physa alexandrina* (Bourg) now known as *Bullinus contortus*. Highly pigmented forms, which he figures as Cercariae of *Amphistomum conicum*, were found associated in the liver of the same snail, with hyaline Cercariae, with pigmented ocular spots, which he describes as Cercariae of *Gastrodiscus aegyptius*.

Total length 0·845 mm. Body 0·474 mm. × 0·427 mm.
Tail 0·40 mm. Form cysts as in 14.

These Cercariae have been found in *Bullinus contortus*, *Planorbis boissyi*, *Limnaea caillaudi*, and *Cleopatra bulimoides*. 1 % *Planorbis*, 5 % *Bullinus* naturally infested.

Fig. 2. Mature redia of 16, showing the anteriorly situated sucker, alimentary canal, germiniferous cells at the posterior end, the numbers of young Cercariae. Size 0·777 mm. × 0·135 mm.

Fig. 3. *Cercaria pleurolophocerca* (Sonsino) found in the majority of specimens of *Melania tuberculata*, from the Zoological Gardens, Cairo. Very striking Cercaria; tail with cuticular caudal expansion.

Total length 1·0 mm. Body 0·27 mm. × 0·08 mm. Tail 0·49 mm.

All Cercariae and Rediae measured, and figured in these plates, have been derived from fresh dissections, and were killed in water by heat.

Fig. 4. Cercaria from liver of *Cleopatra bulimoides*.

Total length 0·807 mm. Body 0·5 mm. × 0·135 mm. Tail 0·300 mm.
(*Cercaria distomatosa*.) Sonsino.
Note the bifid character of the extremity of the tail.

Fig. 5. Cercaria commonly found in *Planorbis*, *Bullinus* and *Physa* (31 % of *Bullinus* naturally infested, and 2 % of *Planorbis*), not described by Looss.

Total length 0·76 mm. Body 0·382 mm × 0·125 mm. Tail 0·387 mm.

Fig. 6. Encysted Cercaria—same as 13—commonly found in liver of *Bullinus*, especially during the winter months. Average diameter 0·165.

Fig. 7. Orange-pigmented Redia of 13. Length 1·43 mm. × 0·143 mm., showing muscular terminal sucker, rudimentary alimentary canal, and contained Cercariae in various stages of development, also lateral appendix.

PLATE V.

Figs. 1 and 2. See legend on plate.

HUMAN INTESTINAL PROTOZOAL AND HELMINTHIC INFECTIONS OBSERVED IN MALTA.

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Lieutenant, attached R.A.M.C.

(A REPORT TO THE MEDICAL RESEARCH COMMITTEE.)

The following observations are the result of two years' examination of the stools of patients invalided to and stationed in Malta. The patients may be roughly divided for convenience into four groups:

(1) Troops sent straight from the Salonika Hospitals to Malta. This section comprised the bulk of the total number of troops examined, and included a few men from Egypt.

(2) Garrison Troops—some not patients—including both British and Maltese. With these are included a few British and Maltese Civilians, connected in some way or another with the Army.

(3) Troops on their way out from England or France. These were mostly Indians and Chinese, who were put off transport at Malta owing to disability.

(4) Prisoners of War interned in Malta.

Altogether 7323 examinations were made of 3370 individual cases.

[1] The author of this paper, the late Lieut. T. Bentham, returned to England at the beginning of 1919, after spending two years in Malta as Protozoologist attached to the R.A.M.C. During his stay in the Island he made many interesting and new observations upon the protozoal and helminthic infections occurring in British troops, natives, and others. As these observations appeared to us to be well worthy of publication, Lieut. Bentham, at our suggestion, undertook to write a report embodying his findings; but, to our deep sorrow, before its completion he fell a victim to the influenza epidemic which prevailed at the beginning of this year. Nevertheless, his unfinished work was found to contain the greater part of his results: and it seemed to us that, incomplete though it was, it recorded observations which ought to be preserved by publication. We have therefore revised his manuscript, to the best of our ability, and charged ourselves with the responsibility of seeing it through the press, as a tribute to the memory of our friend. We would note, however, that the conclusions and opinions recorded in the paper are those of the author himself, expressed in his own words; and that our editorial activities have been strictly limited to the silent correction of obvious slips and errors in the manuscript, and to the elimination of those ambiguities and repetitions inevitable in a report which was merely an unfinished and unrevised first draft. We are indebted to the author's father, the Rev. T. Bentham, and to his sister, Miss Margaret Bentham, for enabling and helping us to put this work on record.

Clifford Dobell.
A. C. Stevenson.

GROUP I.

Group I does not show any features of very great interest. (See Table I.) The percentage of amoebic infections is necessarily very low, because of several important factors which have to be taken into consideration. In the first place an enormous proportion of these patients had suffered from bacillary dysentery in some form or another, and there was no reason to believe that they were otherwise infected. Secondly, and especially towards the end of 1918, a large number of troops had been successfully treated in the Salonika Hospitals, or at least showed a temporary benefit from such treatment. Thirdly, there seems to be, on the part of some medical officers, an insatiate craving for the discovery of animal parasites in every patient. In consequence the stools from patients suffering from such diseases as phthisis, malaria (quinine colitis), influenza, etc., were frequently sent up to the laboratory—some for repeated examination for animal parasites.

Table I.

Troops invalided from Salonika to Malta.

Total No. examined 2485.

Infected with:	No.	%
Entamoeba histolytica	328	13·2
Free Amoebae (not determined)	210	8·5
Other Protozoa	579	27·3
Taenia saginata	1	—
Taenia solium	1	—
Fasciola hepatica	1	—
Trichuris trichiura	8	0·32
Ascaris lumbricoides	9	0·36
Strongyloides stercoralis ...	2	—
Ankylostoma duodenale ...	1	—

In March 1917 an attempt was made to determine the number of amoebic carriers in a certain convalescent camp on the Island. The convalescent dysenterics were confined to a camp of their own, and it was in this camp that a small laboratory was set up for investigation of the stools. Here again the results were somewhat disappointing. There were about 1300 convalescent dysenterics in this camp, but owing to the limited time at our disposal, only 508 cases could be allowed one examination each. This was partly owing to the difficulty of getting the men to provide specimens. Amongst these 508 cases only 52 were found to be carriers of *Entamoeba histolytica*, and there were 64 cases of *Giardia* (= *Lamblia*) infection. This provided the somewhat low percentage of 10·2 for the former. It will be seen from Table I, however, that the total percentage for *E. histolytica* in the Salonika troops is appreciably higher than this, on account of the fact that the majority of individuals were examined more than once each.

In this group worms were of rare occurrence, and the only men of any interest were the two infected with *Strongyloides stercoralis*. One, an officer, had lived in Ceylon and was admitted to hospital for amoebiasis. Examination showed the presence of *S. stercoralis* larvae in large numbers. This officer suffered no ill effects, and on blood examination no appreciable eosinophilia was present.

The other case was a sergeant who was very heavily infected. Extracts from his case sheet may be of interest:

Age 30. Admitted to Hospital from — Battery, to be kept under observation for anaemia. History: Mauritius 1910. Had slight attack of malaria and occasional diarrhoea with blood and mucus at times. Headache, and pain all over body and stomach. Malta 1915. No illness. Khartoum 1916. No illness but severe headaches. At present complains of frontal headache which is continuous, with sudden sharp exacerbations chiefly felt over eye-brows. Tenderness over points of exit of superior and inferior orbital nerves. Suffers from visual troubles manifested by the appearance of zig-zag lines and distortion in shape of objects looked at. Such troubles usually occur during a spasm of pain. Eyesight normal during intervals.

General condition: Thin and anaemic. Left varicocele. No abnormal physical signs in heart, lungs, and alimentary system. Blood count: Red corpuscles 5,920,000. Haemoglobin 45 per cent. Differential leucocyte count: Polymorphs 37 per cent., lymphocytes 30 per cent., large mononuclears 3 per cent., eosinophils 29·5 per cent., and a few mast cells.

In view of the possibility of helminthiasis examination of stool was made, and *Strongyloides stercoralis* and *Entamoeba coli* cysts were found.

Treatment. The patient was instructed to disinfect his hands each time he used pan. He had calomel gr. iv on 14. xii. 17, and gr. v on 15. xii. 17, and the eosinophilia dropped to 14 per cent. Stools swarming with embryos. Thymol treatment as follows: From 18 to 29. xii. 17 Sod. Bicarb. ʒii daily, to clean duodenum of mucus as much as possible. Evening of 28. xii. 17 dose of salts Nothing in way of nourishment. On 29. xii. 17, at 6 a.m., Thymol 20 grs. in capsules; another dose at 7 a.m. and another at 8 a.m. Dose of salts at 10 a.m.; a little black coffee and tea at 11 a.m. During this time the patient was instructed to lie on his right side, and to abstain from taking any fatty substances all day.

5. i. 18. Living embryos still present in stools. Calomel treatment, gr. i t.d.s.

10. i. 18. Still on calomel treatment. Living embryos of *Strongyloides* still being passed.

15. i. 18. Calomel dose changed. To start with calomel gr. v on alternate days.

22. i. 18. General condition not improving: embryos still present. Calomel discontinued.

23. i. 18. Sulphur ʒii t.d.s.

30. i. 18. *Strongyloides* still present in stools. Off sulphur and put on milk treatment, *i.e.*, absolute milk diet and calomel gr. v on alternate days.

11. ii. 18. Embryos still found. Treatment stopped.

Group II.

(See Tables II–V.)

This group may be divided into four sections:

(1) R.A.M.C. and Nursing Staff.

(2) Other Garrison Troops, including Northumberland Fusiliers, West Yorks, A.S.C. and their relatives.

(3) Maltese Garrison Troops.

(4) Civilian employees.

In the second section nothing of importance is to be noted, but the first and third are of no small interest, in that they illustrate the endemic nature of amoebiasis in Malta and its spread among individuals who are more or less compelled to live together under the same conditions and in the same environment.

Table II.

R.A.M.C. Garrison, and Nursing Staff.

Total No. examined 279 (Men 233, Women 46).

Infected with:	No.			%		
	Men	Women	Total	Men	Women	Total
Entamoeba histolytica	33	9	42	14·1	19·2	15
Free Amoebae (not determined)	30	6	36	—	—	12·9
Other Protozoa	107	12	119	—	—	42·6
Oxyuris vermicularis	1	—	—	—	—	—

To illustrate the first section (Table II), the following experiment was made:

N.C.O.'s and men of an R.A.M.C. detachment to the number of 147 were ordered to provide stools for microscopic examination for animal parasites. All these men were of the same company, R.A.M.C., and had been on the Island together for about three years. The members of the Sergeants' Mess, including full corporals, numbered thirty; and of these eleven were found to be infected with *E. histolytica*, giving a percentage of 36·6 on one examination only. Two of the sergeants were on the cooking staff. One of these was found positive during the above routine examination, and the other was negative after repeated examinations, although he was reputed to be a carrier. Subsequently four members of this Mess were admitted to hospital with acute dysentery, although ten members went through an "ambulant" course of treatment with emetine hydrochloride (hypodermically) and emetine bismuth iodide pills. One of these men eventually died of dysentery from a superimposed infection with Shiga's bacillus.

The remaining 117 of this detachment consisted of lance-corporals and privates. Only nine cases, after one examination, were found to be carriers (7·6 per cent.). Two of them afterwards developed acute symptoms though they were all treated by the ambulant method. These 117 men were employed in varied duties, mostly as ward and ablution-room orderlies. The occupations of the nine infected men were as follows—butcher (1), registrar's clerk (1), orderly room clerk (2), ward orderly (3), cook (1), ablution-room orderly (1). The source of infection in four of these cases is fairly obvious, in that the men were employed in close contact with dysentery cases. The registrar's clerk may have obtained his infection through handling case-sheets. Five cooks employed in the same cook-house were infected with *Chilomastix*. Escape from infection by the remaining men is to be attributed to the fact that owing to their duties they probably kept their hands cleaner and were more familiar with the use of antiseptics.

Members of the Nursing Staff, *i.e.*, Sisters, V.A.D.'s, etc., were mostly examined for certificates of health before travelling home through Italy. A few, however, were admitted to hospital with acute symptoms. Some of the carriers developed sub-acute symptoms after the administration of emetine hydrochloride injections and emetine bismuth iodide pills, a condition we have often noticed in the treatment of other cases. The pills sometimes produced violent diarrhoea, with occasional passage of blood and mucus in which no amoebae were present. A similar condition often prevails after treatment with large doses of quinine for malaria.

Forty-six women were examined for infections with *E. histolytica*, and of these nine (19·2 per cent.) were found infected (see Table II).

As will be seen from Table III the remaining Malta Garrison troops gave a very small percentage of infection. They were all admitted with diarrhoea and dysentery, but our records show that this was, in a great number of cases, of bacillary origin.

Table III.

Malta Garrison (1 *G.B.N.F.*, 1 *G.B.W. Yorks, R.E., A.S.C., R.A.F.*).

Total No. examined 124.

Infected with:	No.	%
Entamoeba histolytica	12	9·6
Free Amoebae (not determined)	22	17·7
Other Protozoa	32	25·0

Twenty-seven of the relatives of these garrison troops were admitted to hospital, and of these 24 were females. These, together with the three males, gave a percentage of infection with *E. histolytica* of 22·2 (see Table IV).

Table IV.

British Civilians, including Relatives of Garrison Troops, Civil Clerks, etc.

Total No. examined 27 (Men 3, Women 24).

Infected with:	No.			%
	Men	Women	Total	
Entamoeba histolytica	1	5	6	22·2
Free Amoebae (not determined)	2	4	6	22·2
Other Protozoa	—	9	9	33·3

Section 3 consists wholly of Maltese Garrison troops, and this was the most heavily infected class with which we had to deal (see Table V), 27·5 per cent. being *E. histolytica* carriers. An example, giving the result of examination of the garrison of a small fort on the coast, will serve to show the prevalence of *E. histolytica* infections among the Maltese. One examination only was made in each case. The garrison of this fort, situated on one of the highest points of the island, numbered 74 men, of whom eight were sergeants and corporals. The remainder were bombardiers and gunners and one trumpeter. Most of

the latter group had their quarters in a large circular underground stone building which was clean and well ventilated. Two men lived at home, and a few others were posted to small outlying forts close to the main buildings. Every week-end a certain number of these men were granted leave to go to their homes. Flies were very prevalent in the summer, especially in and about the outlying smaller forts, but chlorinated water was in general use for drinking purposes.

Table V.

Maltese Garrison Troops (1 *and* 2 *K.O.M.R.M., R.M.A., A.S.C.* (*M.T.*), *and R.F.C.*).

Total No. examined 200.

Infected with:	No.	%
Entamoeba histolytica	55	27·5
Free Amoebae (not determined)	30	15
Other Protozoa	102	51
Taenia saginata	3	1·5
Trichuris trichiura	13	6·5
Ascaris lumbricoides	2	1

The sergeants and corporals (eight men) had quarters separate from the rest of the garrison. Their ages ranged from 46 to 27½ with an average of 38, and they were older men than the rest of the garrison. All were negative—none of these men being infected with *E. histolytica* as far as the result of one examination was concerned.

Of the remainder (66 men, average age 27), 23 were infected with *E. histolytica*, giving the remarkable percentage of 34·8 on one examination only. Of these men seven had been associated in military life for the previous three years at a fort on the other side of the island. 16 out of the 66 men were free from parasites of any kind, the remaining 50 having parasites, other than *E. histolytica*, in the following numbers: *Chilomastix* 19, *Lamblia* 11, *E. coli* cysts 20, free amoebae 9, *Trichomonas* 2, *E. nana* cysts 4, *Isospora hominis* 1, Helminths 5, Acarina (*Tyroglyphus*) 3. It is therefore not improbable that, if a sufficient number of examinations could have been made, lasting over comparatively long periods of time, nearly 100 per cent. of this garrison would have been found to be infected with *E. histolytica*.

Several of these men were afterwards admitted to hospital with acute symptoms, and the R.A.M.C. orderly who was stationed at the Fort at the time when the examinations were made, told me that, on going his round of inspection, he often observed blood and mucus in latrines. Apparently, in the majority of cases, the Maltese look upon the passage of blood and mucus as a normal event, which can be easily remedied by the administration of some form of tannic acid. The methods of feeding employed by the majority of the town class Maltese leave much to be desired, and large quantities of faecal matter must be ingested together with their food. House-flies are very abundant during the hot weather in Malta; and I have often observed children

with their eyes and mouths black with flies, no attempt being made to drive them away. It is not surprising, therefore, that the Maltese are so heavily infected with all kinds of animal parasites. The comparative frequence of worm-eggs in the stools of this people also serves as an illustration of this condition.

Table VI.

Maltese Civilians, Batmen, Cooks, etc.

Total No. examined 78.

Infected with:	No.	%
Entamoeba histolytica	14	18
Free Amoebae (not determined)	7	9
Other Protozoa	36	46·1
Taenia saginata	1	1·3
Hymenolepis nana	1	1·3
Trichuris trichiura	2	2·5
Ascaris lumbricoides	2	2·5

Section 4 of this Group (see Table VI) comprises Maltese civilians who were, for the most part, prospective cooks and waiters to be employed by the military. These included the servants of one household and of one mess. They were all of a better class than the average Maltese militiaman or gunner, and were not so heavily parasitized. Owing to their occupations, they had better housing, were obliged to be more cleanly in their habits, and lastly, were accustomed to better food. It is no exaggeration to say that *E. histolytica* and worm-eggs were nearly always found in the dirtier and more slovenly people. I think there were three exceptions. 78 individuals were examined with a percentage of *E. histolytica* cases of 18. None of these to my knowledge had suffered from acute symptoms. The servants of the household mentioned above were ten in number (together with three Maltese and two British soldiers included in Table V as garrison cases). On being questioned every one of them denied having had diarrhoea at any time (*sic*). Of these 15 servants, eight were infected with animal parasites, three of them with *E. histolytica*. The occupations of the three were respectively caretaker, messenger, and housemaid.

The servants in the Officers' Mess were seven in number. Four had animal parasites, and only one of them *E. histolytica*.

Group III.

This Group (Table VII) consisted mainly of Indian and Chinese troops who were proceeding home from France. The Indians mostly belonged to Labour Corps, and a fair proportion came from the province of Bihar. There were two Burmese included in this section, and a few men from the Naga Hills. The remainder came from various other parts of India. The Chinese consisted of French Colonial Tirailleurs and Labour Corps. Most of them came from Tonkin. In addition to these a few other French Colonial Troops were admitted to Hospital.

Table VII.

Native Labour Corps, etc. (Indians, Chinese, etc.).

Total No. examined 83 (Indians 61, Chinese 18, Somalis 3, Egyptians 1).

Infected with:	Indians	Chinese	Somalis	Egyptians	% (total)
Entamoeba histolytica	9	5	—	—	16·9
Free Amoebae (not determined)	4	1	—	—	6·0
Other Protozoa	18	—	1	—	22·9
Taenia saginata	1	2	—	—	3·6
Hymenolepis nana	1	—	—	—	1·2
Clonorchis sinensis	—	4	—	—	4·8
Schistosomum haematobium ...	—	—	—	1	1·2
Schistosomum japonicum ...	—	3	—	—	3·6
Trichuris trichiura	14	6	—	—	24·0
Ascaris lumbricoides	9	4	—	—	15·6
Strongyloides stercoralis ...	—	1	—	—	1·2
Ankylostoma duodenale ...	29	6	—	—	42·2

These men contained a large number of animal parasites, and were, for the most part, infested with worms of various kinds. In all, seven different species of worms were identified from their faeces. The percentage infected with *Ankylostoma* was 42·2 in 83 men examined. The infections with *E. histolytica* gave a fairly low percentage, due no doubt to the comparatively clean methods of feeding adopted by the majority of Indians and Chinese. Yet against this, we have a high percentage of infection with *Trichuris trichiura*, suggesting the faecal contamination of food.

Amongst the findings may be mentioned a case of infection with *Balantidium coli*. The patient, an Indian Christian from Bihar, and belonging to a Labour Corps, was admitted to hospital with coryza and a slight temperature. He had no diarrhoea, but in accordance with custom his stool was sent up with those of the other Indians for the usual examination. It was found to contain *E. histolytica* cysts, *Ankylostoma* eggs, and *Balantidium coli* in large numbers. The patient, who spoke perfect English, was questioned as to whether he had at any time suffered from diarrhoea or dysentery, and answered in the negative. He stated that he had always enjoyed perfect health and had been employed as a labourer in his own country. He had never had anything to do with pigs. Thymol grs. xl was given for the *Ankylostoma* infection, and during the administration of this drug the *Balantidium* temporarily disappeared from the stool, to reappear later, when the treatment was over. Emetine given for the *E. histolytica* infection had no effect upon the balantidia. The patient was eventually discharged cured of his *Ankylostoma* infection, but with the balantidia as numerous as ever.

The case just mentioned belonged to a party of sixteen of the Bihar Labour Corps. All these men were infected with *Ankylostoma* in small numbers and were eventually discharged negative, after the customary dose of forty grains

of thymol. Sometimes the dead worms were found in the stools, usually in a state of decomposition.

The largest infection with *Ascaris* occurred in a labourer from Burma. The eggs of this worm were found in the stool in thousands, and so santonin grs. iii was given. I actually counted out 55 adult round worms, passed from time to time, and the isolation orderlies told me that at least another 50 worms were thrown away in a decomposed condition. All these worms were expelled dead, after a single treatment, and no more eggs appeared in the stool. This man was also infected with *E. histolytica*, *Ankylostoma*, and *T. trichiura*.

Multiple infections were more common among the Chinese, and three examples of this may be given for illustration. These three men were French Colonial Tirailleurs, or Riflemen. Their infections were as follows:

(1) *Ascaris*, *Ankylostoma*, *Schistosomum japonicum*, *Trichuris trichiura*, *Clonorchis sinensis*, and *Entamoeba histolytica*.

(2) *Ascaris*, *Ankylostoma*, *Schistosomum japonicum*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Taenia saginata*, and *Entamoeba histolytica*.

(3) *Ascaris*, *Trichuris trichiura*, *Schistosomum japonicum*, *Ankylostoma*, and *Entamoeba histolytica*.

This last case was that of a Chinaman with cerebro-spinal meningitis and diphtheria.

Autopsy: Body wasted. Excess of fluid at base of brain. A little flaky lymph in region of 4th ventricle and fissure of Sylvius. All superficial vessels congested. Slight excess of fluid in 3rd and lateral ventricles. Choroidal plexus congested. No pleurisy. Both lungs congested and oedematous at their bases. Nothing abnormal found in larynx, trachea, or bronchi. Heart, nothing abnormal discovered. Abdomen: Portal veins and principal branches dissected out. No *Schistosomum japonicum* found. Congestion of mucous membrane of colon, extending to upper part of rectum. Three or four small discrete ulcers, apparently amoebic, in caecum and ascending colon. A fair number of *Ankylostoma duodenale* (worms) recovered from washings of contents of duodenum and jejunum; none found attached to mucous membrane. A large number of ecchymoses found on mucous membrane of duodenum and jejunum. Worms all contained blood. One alive, others dead. A great tangled mass of *Trichuris trichiura* and one *Ankylostoma* were obtained from washings of contents of caecum. No ascarids found. (Patient had been treated, and during life had vomited up four ascarids and passed another twenty *per rectum*.)

Cause of death: Cerebro-spinal Meningitis, Amoebiasis.

Another interesting case is that of an old Chinese labourer, age not ascertained, who was admitted to hospital with "P.U.O." (Pyrexia of unknown origin.) Stool examination disclosed an immense number of *Clonorchis sinensis* eggs and nothing else. Nothing otherwise abnormal was detected, and the man died a fortnight after admission.

Autopsy: Liver small and shrunken, covered all over with small excrescent thickened areas, lighter in colour than the normal liver tissue. There was a large primary carcinoma present the size of an orange. The gall-bladder was full of *Clonorchis sinensis*, and on making a small cut into any part of the surface of the liver, two or three flukes emerged. It was calculated that there were about 5000 of these organisms in the liver tissue alone. The pancreas contained a few flukes and its surrounding mesenteric glands were much enlarged. The flukes which were taken from the gall-bladder were green in colour and contained no blood, but those from the liver were full of blood and fleshy in colour. They measured 14–21 mm. in length by 4–5 mm. in breadth. A large number of the parasites were examined with a view to finding out whether there was also an infection with *Opisthorchis felineus*, but all the worms were found to be *Clonorchis sinensis*. Sections of liver tissue showed carcinomatous thickening of the burrows in which the worms lived, surrounded by a small amount of normal liver tissue. Numerous eggs of this fluke were found in the burrows and in spaces in the new tissue. Unfortunately, only a small piece of the liver was kept, but a fair number of flukes were preserved, some of which are now in the Wellcome Bureau, London.

It is to be noted that a large number of these cases suffering from Helminthiasis showed an appreciable eosinophilia but rarely—especially patients who had an *Ankylostoma* infection. It is true that a large number of the men were not very heavily infected, but this blood condition was not evident in the Chinese mentioned above, nor was it noticeable in the Burmese soldier with the large *Ascaris* infection.

The variation of an eosinophil count is well shown by the following table compiled from films made on successive days from the patient, infected with *Strongyloides stercoralis*, already described as a member of Group I (p. 73).

Date	Polymorphs %	Lymphocytes %	Large mononuclears %	Eosinophils %
14. xii. 17	37·5	30	3	29·5
15. xii. 17	30·3	28·7	26·7	14·3
16. xii. 17	42·5	37·5	6·5	13·5
17. xii. 17	40·7	18·3	37·7	8·3
18. xii. 17	51·6	24·6	11·3	12·5
19. xii. 17	41·9	12·7	28	17·4
20. xii. 17	56·7	19·8	7·5	16
21. xii. 17	52·1	19	12·5	16·4
29. xii. 17	26	20	43	11
10. i. 18	31·9	12·4	50·7	5
13. ii. 18	59	20	8	13

In this series of counts there seems to have been a drop in the number of eosinophils after two doses of calomel on 14 and 15. xii. 17, but the count appreciably rises again until thymol was given on 29. xii. 17. It dropped to only 5 per cent. 11 days after this treatment. The other methods of treatment tried during the period from 5. i. 18 to 11. ii. 18—when treatment was stopped —have already been noted on p. 74. During the whole of this time embryos of *Strongyloides* were as numerous as on the first examinations.

GROUP IV.

[The account of this Group, which comprises Prisoners of War interned in Malta, was still unwritten at the time of the Author's death. All the information available is contained in Table VIII.]

Table VIII.

Prisoners of War (German, Turkish, etc.) Internment Camp.

Total No. examined 94 (Germans 68, Turks 20, Greeks 4, Egyptians 2).

Infected with:	Germans	Turks	Greeks	Egyptians	% (total)
Entamoeba histolytica	14	4	—	—	19·1
Free Amoebae (not determined)	12	4	1	—	18·0
Other Protozoa	30	11	1	—	44·7

ON A NEW SACCHAROMYCETE *MONOSPORELLA UNICUSPIDATA* GEN. N. NOM., N. SP., PARASITIC IN THE BODY CAVITY OF A DIPTEROUS LARVA (*DASYHELEA OBSCURA* WINNERTZ).

By D. KEILIN, Sc.D.

(*From the Quick Laboratory, University of Cambridge.*)

(With Three Text-figures.)

The genus *Monospora* (= *Monosporella* renamed[1]) was founded by Metschnikoff in 1884 to designate a parasitic fungus which he discovered in *Daphnia magna*. This monocellular fungus, of which he described only one species, *Monospora bicuspidata*[2], lives free in the body cavity of its host, where it multiplies actively by budding in a yeast-like manner (Fig. I, 1, 2, 3).

When the body cavity of the host is entirely invaded by the parasites, these grow in size, become elongated, and form club- or sausage-shaped asci in each of which is developed a single needle-like spore having both ends pointed (Fig. I, 4, 5 and 6). When the parasitized host dies, it is filled with ripe spores, and healthy Daphnias, which feed on the detritus of their dead and diseased fellows, become infected by ingesting the asci. The latter, when they enter the host's alimentary canal, set free the needle-shaped spores which perforate the gut wall and penetrate in the body cavity (Fig. I, 8) where they germinate laterally, thus starting the new infection (Fig. I, 7).

Metschnikoff's studies on this parasite afford a striking instance of the phenomenon of phagocytosis. In his lectures on inflammation (1893, pp. 83–84), it is stated that directly the spore "appears outside the intestinal wall, it is attacked by leucocytes, which are carried to the spot by the blood-stream. The cells fix themselves on the spore, forming around it a collection of cells, which often fuse together into a plasmodium, which causes the spore to undergo a series of remarkable changes. On being enclosed in the leucocytes the spore

[1] The generic name *Monospora*, given by Metschnikoff to the parasite, is invalidated for the reason stated in the Appendix to this paper, p. 90.

[2] The species of *Monospora*, discovered by Metschnikoff, was described by him under the name *M. bicuspidata*, and under this name it is referred to in his various publications, nevertheless all the authors I have consulted (Zopf, Hansen, Dangeard, Guillermond, Lafar, Saccardo) wrongly name the species *M. cuspidata* Metschnikoff. I do not know who changed the specific name, but incline to the view that the error may have arisen through a misprint or misquotation, none of the authors mentioned having apparently referred to Metschnikoff's original papers.

first loses its regular contour, becomes sinuous, and finally breaks up into a mass of brownish granules...." By isolating infected *Daphnia*, Metschnikoff succeeded in restoring them to health "thanks to the destruction of the spores by their phagocytes. If on the other hand the phagocytic action is inadequate, owing to the continued increase in the number of spores swallowed or for any other reason, the latter begin to germinate and give rise to budding conidia."

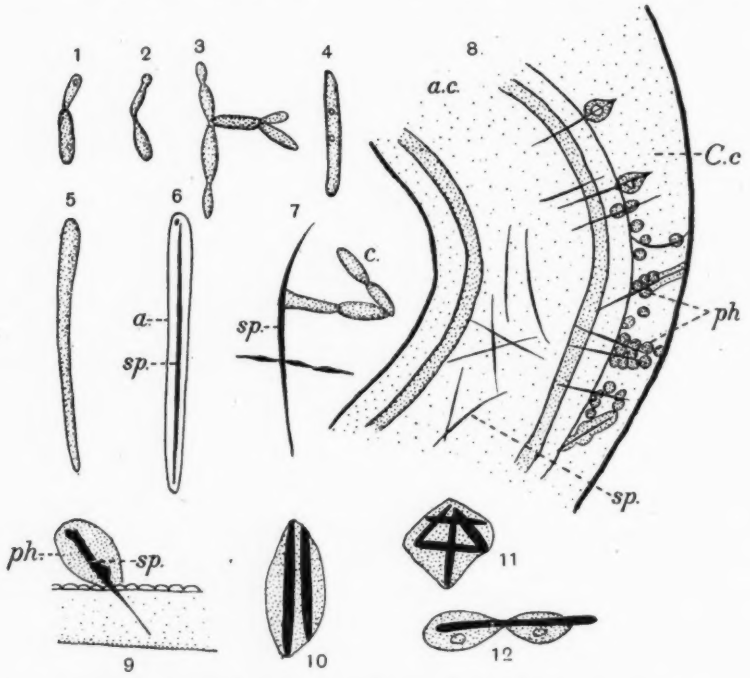

Fig. I. *Monosporella bicuspidata* (Metsch.), after Metschnikoff, slightly schematized. 1, 2 and 3, budding cells; 4 and 5, elongated cells developing into asci; 6, ascus (*a.*) with spore (*sp.*); 7, germination (*c*) of a spore (*sp.*) that has penetrated into the body cavity of the host; 8, anterior portion of *Daphnia* showing the spores (*sp.*) of *M. bicuspidata*, free in the alimentary canal (*a.c.*) or perforating its wall and penetrating into the body cavity (*C.c.*) where they are surrounded by phagocytes (*ph.*); 9, spore (*sp.*) penetrating into the body cavity, partly surrounded and digested by a phagocyte (*ph.*); 10, phagocyte containing two cells of parasite; 11, plasmodium of phagocytes containing several cells of *Monosporella*; 12, fungus cell surrounded by two phagocytes.

Following upon Metschnikoff, almost all authors (Zopf 1890, Hansen 1904, Dangeard 1907, Guillermond 1907, Lafar 1910 and Saccardo 1911) dealing with *Monospora* refer to Metschnikoff's observation. Chatton (1907), however, seems to be the only author who has actually seen the parasite; thus, in his general account of parasites and commensals living upon Cladocera he mentions (p. 807) that every year during the spring he used to find *Daphnia*

in abundance parasitized by *M. bicuspidata*. His material was derived from a tank at the Jardin des Plantes, Paris.

The genus *Monospora*, or, as we shall call it now, *Monosporella*, has hitherto comprised: (*a*) the species *bicuspidata* Metschnikoff and (*b*) a yeast-like fungus found by Bütchli (1876, p. 148, Pl. XIV, fig. 8) in the coelom of a free-living nematode, *Tylenchus pellicidus* Bast. Unfortunately this author's description and figures are insufficient for determining more than the genus to which the parasite belongs.

This summer (1919) I found a new species of *Monosporella*, which I propose to name *Monosporella unicuspidata*, living in a Dipterous larva: *Dasyhelea obscura* Winnertz[1]. The larvae of this Ceratopogonid live usually in the thick brown sap which fills the infected wounds of elm or horse-chestnut trees. Whilst larvae collected from the wounds of a horse-chestnut, standing on the grounds behind the School of Agriculture, Cambridge, harboured *Monosporella*, those taken from elms (at Newnham and along the Backs, Cambridge) were not infected by this fungus, they contained however other parasites which will be dealt with separately.

The proportion of larvae infected with *M. unicuspidata* appeared to be low, for but twenty out of several hundred forming the material examined by me were found to be infected. The actual proportion of infected individuals doubtless varies in nature and it must have been greater in this instance. Owing to the larvae being insufficiently transparent to permit the detection of the few parasites that may occur in mild forms of infection, some of these doubtless escaped notice. It appears probable, moreover, that a number of larvae may rid themselves of parasites by phagocytosis as some examples of *Daphnia* do when attacked by few *M. bicuspidata*. The parasitized larvae observed by me belonged to three successive generations of *Dasyhelea* and they were all heavily infected. A parasitized *Dasyhelea* larva is easily recognized by the milky appearance of the body and especially of its posterior segments. Examined microscopically, the larva shows an enormous number of elongated refractive cells, completely filling the body cavity, and in some cases so crowded together that they all take a direction parallel to the long axis of the body of the larva. In spite of the great number of parasites that are present, the larvae are able to move, the fat body seems to be the only organ which is completely destroyed, whereby the larva becomes more transparent and the parasites are better observed. Finally the larva dies and decomposes rapidly, thus setting free the resistant forms of the parasite. In the living larva, even when heavily infected, almost all developmental stages of the parasite are easily seen by cutting open the larva in a drop of normal salt solution or Amman's lactophenol[2].

[1] The identification of this Ceratopogonid I owe to the kindness of Mr F. W. Edwards of the British Museum.

[2] Amman's lactophenol, pure or mixed with 0·5 % of cotton-blue, which was used for the examination of the parasite, is highly recommended for similar purposes.

Description of *Monosporella unicuspidata* n. sp. (Fig. II, 1 to 17).

In the young stages, the parasite occurs in the form of small oval cells from 4μ to 10μ long, budding at one end. The buds are usually single (Fig. II, 1 to 6) but occasionally two or three buds are formed simultaneously (Fig. II,

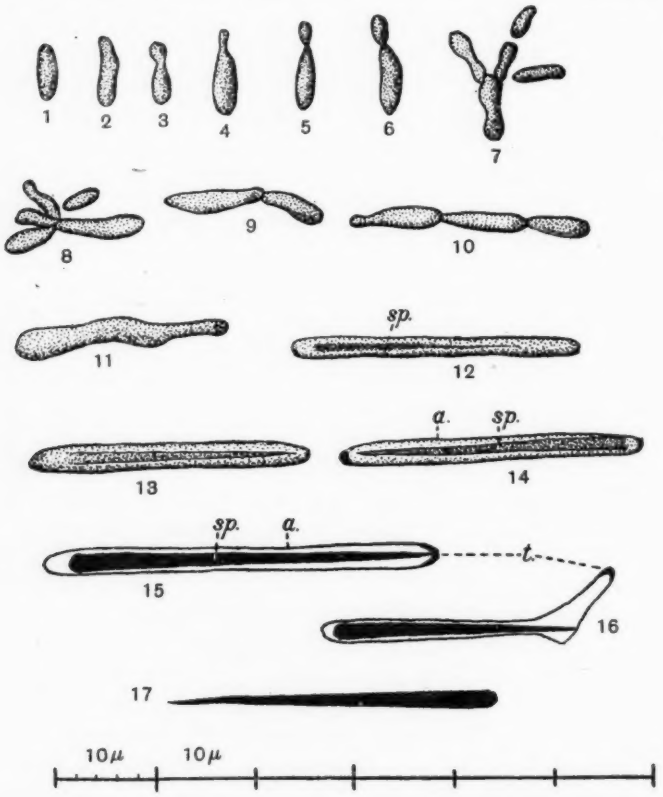

Fig. II. *Monosporella unicuspidata* n. sp. 1 to 6, different stages of budding cells; 7 and 8, rare cases of multiple budding; 9, ordinary budding; 10, chain with three cells; 11, elongated cell developing into ascus; 12, ascus with the beginning of the spore formation (*sp.*); 13 and 14, more advanced stages of spore formation; 15, ascus (*a.*) with well formed spore (*sp.*), *t.* thickened wall of the ascus; 16, deformed ascus; 17, spore. Figures drawn with camera lucida, slightly schematized, the refractory spores being represented in black. The scale of magnification is given beneath the figures.

7, 8). The new buds generally detach themselves very soon and begin to bud; in only a few cases did I find three cells joined to form a chain 24μ in length (Fig. II, 10). When the body of the larva is completely invaded, the parasites become elongated and acquire a uniform shape reaching 30μ in length and

2·5μ in width (Fig. II, 13, 14). These elongated forms correspond to asci and are at first transparent having finely granulated protoplasm. As development proceeds, the protoplasm near one end of the ascus begins to show a small triangular refractive body which gradually elongates until it occupies almost the whole length of ascus. The refractive body (Fig. II, *sp.* 14, 15) gradually becomes clearly defined and finally develops into a long needle-shaped unicellular spore with one end sharply pointed, the other end truncated. The space between the spore and walls of the ascus is filled with transparent fluid, and the end of the ascus facing the pointed portion of the spore is thickened. In some cases, after the death of the larva, when the asci escape into the fluid surrounding the insect, the ascus walls become deformed so that the thickened end is bent to one side of the spore (Fig. II, 16). The asci and spores vary in size, the asci measure 30μ to 40μ and the spores 24μ to 35μ in length; the truncated end of the spore usually measures 1·8μ across.

I have failed to observe the liberation of the spores from the asci and the germination of spores. In only one larva was the alimentary canal found to contain several free spores (Fig. II, 17), but unfortunately the larva was damaged during examination whereby further observation was precluded.

As the spores of *M. unicuspidata* have only one end pointed, whilst *M. bicuspidata* (Metsch.) has spores with both ends pointed, it is probable that the first named species has a poorer chance of perforating the alimentary canal of its host and this may account for the smaller proportion of infected hosts as compared to what has been observed with *M. bicuspidata* and *Daphnia*.

It is worthy of note that other Dipterous larvae (those of *Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meig., *Aulacogaster rufitarsis* Mcq., *Phaonia cincta* Zett. and a few Eristalines, Drosophilids and Dolichopodids) living under the same conditions as *Dasyhelea obscura*, were not found to be infected with *Monosporella unicuspidata*.

The genus *Monosporella*, hitherto known as *Monospora* Metschnikoff, is often placed by systematists near to the genus *Nematospora* Peglion (Fig. III). The latter, which contains but one species *N. coryli*, discovered and named by Peglion (1901), is a parasite of the hazel-nut in Italy. It is a budding, yeast-like fungus with elongated cells; the ascus is sausage-shaped 65μ–70μ long by 6μ–8μ broad, and contains 8 spores in two longitudinally disposed bundles of 4, separated by an interval midway along the length of the ascus. These spores are elongate spindle-shaped, with a long flagellum at one end and measure 38μ–40μ without the flagellum which is about 35μ to 40μ long. Before germination, the spore loses its flagellum and broadens. Peglion succeeded in cultivating *N. coryli*, finding that it grew well on sterilized sugar-beet or meat-broth gelatin and badly in fluid media, where it formed only a mycelium.

The systematic position of the genera *Monosporella* and *Nematospora* is not yet clearly defined. Metschnikoff and Zopf placed *Monosporella* among

the true yeasts, and Peglion divided the Saccharomycetes into four genera, *i.e. Saccharomyces, Schizosaccharomyces, Monospora* (= *Monosporella*) and *Nematospora.*

On the other hand Hansen (1904), to whom we owe the recent classification of Saccharomycetes which is accepted by almost all mycologists, considers the last two genera as representing "doubtful Saccharomycetes," and he remarks that they are rather rare fungi which were observed only by the authors who discovered them[1], moreover, that he and his collaborators had searched for them in vain in the hope of obtaining material for purposes of study. Notwithstanding the great authority of Hansen, I cannot but

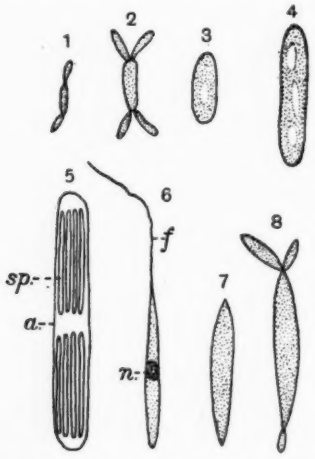

Fig. III. *Nematospora coryli* Pegl. after Peglion. 1 and 2, budding cells; 3–4, stages of growing cell; 5, ascus (*a.*) with eight spores (*sp.*); 6, spore stained with gentian violet, showing the nucleus (*n.*) and flagellum (*f.*); 7, spore after losing its flagellum; 8, budding spore.

believe that *Monosporella* and *Nematospora* have been sufficiently known for purposes of classification, and that they should without hesitation be placed in the family of Saccharomycetaceae. In fact all the characters of this family, as defined by Hansen himself, cover perfectly well the representatives of these two genera. Thus Hansen's classification, with the few modifications and details added by Lafar (1910), is as follows:

I shall omit the expressions (for the group A) "true Saccharomycetes" and (B) "doubtful Saccharomycetes" and shall add more details bearing on the genus *Monosporella.* In Hansen's classification the Saccharomycetaceae are widely separated from the genus *Schizosaccharomyces* for which he creates the family Schizosaccharomycetaceae.

[1] I would note that *M. bicuspidata* Metsch. was often observed by Chatton (1907) and cultures of *N. coryli* were sent by Peglion to the late Prof. A. Giard of Paris who tried to inoculate hazelnuts with the fungus.

Family **Saccharomycetaceae.** Monocellular, sporogenic budding fungi. Each cell is a potential sporogenic cell (or ascus). The number of spores in the ascus is usually 1 to 4, seldom 12. The spores are monocellular. Typical mycelium is formed by a few species.

A. Spore rounded, oval, pileate or lemon-shaped with or without projecting rim.

I. GROUP. In Saccharine nutrient liquids they furnish only sedimental yeast at the outset, surface films occur only at a later period, if at all. Films more or less mucilaginous. Spore smooth, globular or oval, with 1 or 2 membranes. Spore germinates either by gemmation or by producing a promycelium. Great majority produce alcoholic fermentation.

Genus 1. *Saccharomyces* Meyen. Spore with single membrane; germinates by ordinary gemmation. In addition to yeast cells a few of them produce mycelium with well defined septa.

Genus 2. *Zygosaccharomyces* Barker. Ascus is formed after cell fusion.

Genus 3. *Saccharomycodes* Hansen. Spore with a single membrane, germinates into a promycelium, the new cells, being incompletely separated, form a mycelium with well defined septa.

Genus 4. *Saccharomycopsis* Schiönning. Spores with two membranes.

II. GROUP. Film produced on the surface of nutrient solution immediately after the same has been inoculated. The film has a dry dull appearance due to the inclusion of air bubbles. Several species produce esters, and a few of them do not cause fermentation. Spore with a single membrane, of different shapes with or without projecting edge.

Genus 5. *Pichia* Hansen. Spore rounded, hemispherical or irregular and angular. A strong mycelium is formed. No fermentation.

Genus 6. *Willia* Hansen. Spore pileate, or lemon-shaped, with a projecting rim. Most of the species possess considerable ester-forming powers, but a few do not produce fermentation.

B. Spores acicular or spindle-shaped, parasitic fungi.

Genus 7. *Nematospora* Peglion. Elongated budding cells; sausage-like ascus containing eight unicellular spores in two bundles of four. Spore elongated, spindle-shaped with a long flagellum, which is lost before germination. Parasitic on hazel-nuts in Italy. Grows well on sugar-beet or meat-broth gelatin, badly in liquids where it forms mycelium only. One species: *N. coryli* Peglion (Fig. III).

Genus 8. *Monosporella* (= *Monospora* Metschnikoff 1884). Budding, yeast-like fungi, each cell a potential ascus. The latter is elongated and

produces one unicellular acicular spore. Parasite in the blood of invertebrates. Not yet cultivated. The genus comprises the following species:

(1) *M. bicuspidata* (Metschnikoff 1884). Asci slightly narrowing at one end; spores pointed at both ends. Parasite in the body cavity of *Daphnia magna* (Crustacea) (Fig. I).

(2) *M. unicuspidata* n. sp. Asci of elongated but regular form with the wall thickened at one pole. Spore pointed at one end, truncated at the other. Parasite in the body cavity of Ceratopogonid larvae: *Dasyhelea obscura* Winnertz. (Insecta: Diptera.) (Fig. II.)

(3) *M.* sp. Yeast-like fungi found by Bütschli (1876) in the coelom of a free-living nematode: *Tylenchus pellicidus* Bast. (Vermes.)

(4) *M.* (?) sp. Yeast-like fungi of elongated shape found by Caullery and Mesnil (1899 and 1911) in a Polychaete worm *Potamilla torelli*, where they seem to produce a special kind of tumour ("néoformation papillomateuse"). They consider this yeast to be related to *Monospora* although they did not succeed in finding the spores. They also mention a similar yeast occurring in a pelagic Copepod *Acartia*.

Family **Schizosaccharomycetaceae.** Endosporogenic, monocellular fungi, reproduce by fission which is preceded by the formation of a septum that at once commences to divide into two lamellae from outside. No budding occurs. Spores unicellular of which 1–8 occur in each ascus. In some cases formation of asci is preceded by fusion. Spores stained blue by a solution of iodine in potassium iodide. The cells never contain glycogen (a contrast to Saccharomycetaceae). Produce alcoholic fermentation; one genus *Schizosaccharomyces* Lindner.

Acknowledgment.

I am much indebted to Professor G. H. F. Nuttall for valuable suggestions in connection with this study.

APPENDIX.

Concerning the re-naming of Metschnikoff's genus Monospora.

According to Lafar (1910, p. 292), "the genus *Monospora* Metschnikoff ought really to be re-named, since this title has already been applied by Hochstetter, to one of the Flacourtiaceae."

This statement is correct. I would add that Hochstetter's name dates from 1841. However, in Warburg's monograph of the Flacourtiaceae (1894, in Engler and Prantl's *Die natürlichen Pflanzenfamilien*, Part III, p. 37) I find that the name *Monospora* Hochstetter is condemned as a synonym of *Trimeria* Harvey (1838).

On the other hand I find that the name *Monospora* was also given by Solier in 1845 to an Alga of the family Rhodomelaceae; the name was accepted by all specialists of the group and is actually quoted in botanical text-books.

The foregoing evidence serves unfortunately to condemn Metschnikoff's name *Monospora* as being preoccupied. This is regrettable because the name has long been associated with that author's early observations on the important phenomenon to which he gave the name of phagocytosis. I have endeavoured, however, to replace the original name by another which expresses the same meaning, and *Monosporella*, which I propose, seems most fitting, other suitable names being preoccupied.

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ON THE OCCURRENCE OF A SUPPLEMENTARY CHROMATIC BODY IN *MAUPASELLA NOVA* CÉPÈDE (CILIATA ASTOMA), AN INTESTINAL PARASITE OF EARTH-WORMS (*ALLOLOBOPHORA CALIGINOSA* SAVIGNY).

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(With Plate VI.)

Maupasella nova Cépède was discovered by Maupas (1877) in the alimentary canal of an Algerian earth-worm and subsequently described by Cépède (1910). As was well remarked by the latter author, this ciliate varies much in size and in form, long specimens measuring 80–95 by 25μ, and short ones only 50–75 by 27–47μ. The essential characters of *Maupasella* are: (1) the presence of an anterior fixing apparatus in the form of a conical process derived from thickened ectoplasm, (2) an elongated ribbon-like macronucleus, (3) irregularly disposed contractile vacuoles, and (4) dense ciliation. Moreover, the micronucleus (Pl. VI, fig. 18 and *m* in all the other figures), which is often difficult to see, is spindle-shaped, with its axis parallel to that of the ciliate's body, strangulated in the middle and with the chromatin condensed into a disc lying in the strangulated portion of the spindle.

In many specimens of *M. nova* obtained from the alimentary canal of *Allolobophora caliginosa* Sav. collected near Paris (France), I found a ribbon-like supplementary chromatic body which I propose to describe here. These bodies occur in specimens of *Maupasella* which are of different size and form. They stain deeply and uniformly with basic stains, especially with iron-hematoxylin, and never show the granulated structure which is so characteristic of the macronucleus. Their position in the endoplasm of the ciliate, as well as their shape, varies very much (Pl. VI, figs. 1–17); in some specimens of *Maupasella* the chromatic body is straight and parallel to the macronucleus, while in others it is curved in the form of an S or C, or is strangulated in the middle and having one end twisted round the macronucleus (Pl. VI, figs. 9, 10 and 12), but in no case has any continuity between the latter and the chromatic body been observed. I nvery elongated examples of *Maupasella*, which are going to divide (Pl. VI, figs. 9 and 10), and in already dividing specimens

(figs. 11, 12, 13, 14 and 17), the chromatic body always lies in the posterior portion of the ciliate, so that after the division, this body is inherited by the posterior individual, while the anterior is always devoid of it. This shows that the chromatic bodies of different specimens of *Maupasella* are in no way related to each other, but are formed independently in each ciliate, which possesses them.

In spite of a great number of *Maupasella* having been found to contain the chromatic body, I have not yet been able to trace its origin and all I can do at present is to discuss critically several explanations which might be offered regarding its nature.

(1) The absence of any structure or stages of reproduction in this body, as well as its great polymorphism, render very doubtful the supposition that it represents an unknown parasite of ciliates.

(2) It might be supposed that it is derived from the macronucleus of the ciliate by longitudinal division. The occurrence of a double macronucleus is already known in ciliates of the family Anoplophryinae. It was first described by Schneider (1892) in *Hoplitophrya* sp., an intestinal parasite of a fresh-water Oligochaete, among several specimens of which he found 3 or 4 individuals with two macronuclei. These abnormal ciliates showed an ordinary multiple or catenular process of segmentation during which both macronuclei underwent a similar division.

Double macronuclei were also observed by Léger and Duboscq (1904, p. 343) in *Anoplophrya brasili*, an intestinal parasite which they discovered in *Audouinia tentaculata* Mont. As to the origin of the two parallel macronuclei, they do not agree with Schneider (1892), who supposed them to arise from an abnormal conjugation: they explain them, on the other hand, as a result of an ordinary longitudinal fission of a single macronucleus.

But even if we admit the occurrence of longitudinal cleavage of the macronucleus in Anoplophryinae, this process will not explain the origin of the supplementary chromatic body in *M. nova*, for the following reasons: (*a*) the form and structure of the chromatic body differ very much from those of the macronucleus, (*b*) there is no continuity between these two structures, and (*c*) the chromatic body never undergoes segmentation when the macronucleus divides.

(3) The chromatic body does not originate from the micronucleus, as in almost all specimens of *Maupasella* which contained it, the micronucleus was normally developed.

(4) Its origin cannot be interpreted as a result of abnormal conjugation, as this process of reproduction has not yet been observed in Anoplophryid parasites of earth-worms.

(5) It could be suggested also that this body is related to the so-called spicule, an endoplasmic rigid body which normally exists in other Anoplophryinae parasitic in earth-worms, namely *Mesnilella secans* Stein, *M. clavata* Leidy, *M. spiculata* Warpachowsky and *M. fastigata* Möbius. Unfortunately

we know very little of the structure and development of the spicule, which according to Möbius (1888, p. 104) stains well with osmic acid and safranin.

(6) According to Fauret-Frémiet (1907, quoted by Cépède, 1910, p. 500) the endoplasm of *Anoplophrya striata* contains mitochondrial bodies. The existence of extranuclear chromatin was, on the other hand, mentioned by Cépède in *Anoplophrya alluri* and *Herpetophrya astoma* Siedlecki. The endoplasm of *M. nova* undoubtedly contains diffused chromatin as it stains usually with basic stains. Very often the extranuclear chromatin is more condensed into two lateral bands parallel to the macronucleus (Pl. VI, figs. 8, 10, 11 and 14). It is possible that the supplementary chromatic body is the result of a further stage of condensation of the extranuclear chromatin, produced under the influence of some change in the external conditions of life of the ciliate, in this case, in the alimentary canal of the host.

I do not think that a similar supplementary chromatic body has yet been described in other ciliates, and I hope that further observations on similar abnormalities in other parasitic or free-living species will help to elucidate the origin and the nature of this structure.

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EXPLANATION OF PLATE VI.

MAUPASELLA NOVA CÉPÈDE.

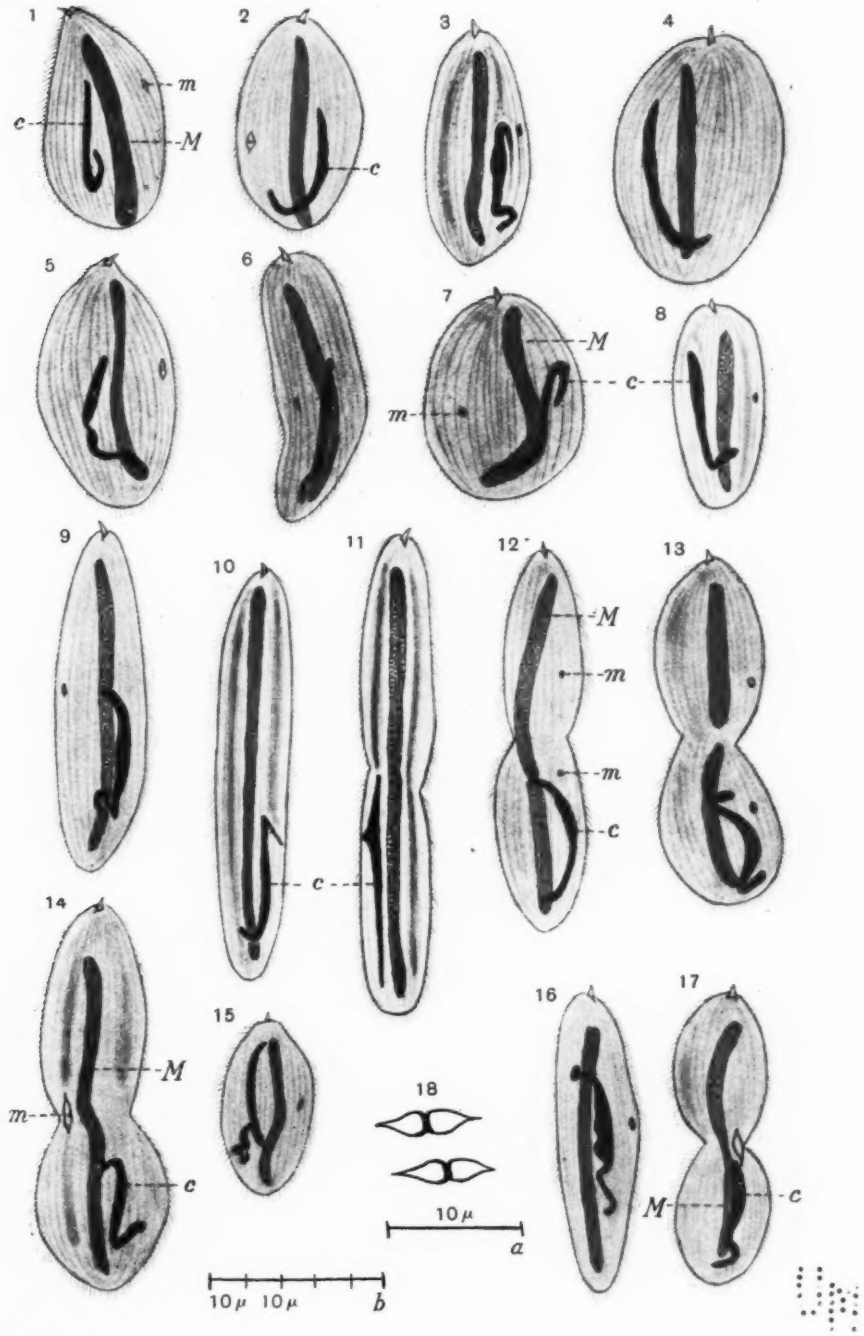
c. supplementary chromatic body; *M.* macronucleus; *m.* micronucleus. *a.* scale of magnification for all the figures except fig. 18; *b.* scale for fig. 18.

Figs. 1–9 and 15, 16. Different forms of the supplementary chromatic body in *M. nova*.

Fig. 10. Elongated form of *M. nova* showing the chromatic body occupying the posterior portion of the ciliate.

Figs. 11–14 and 17. Dividing stages of *M. nova* with the supplementary chromatic body localized in the posterior individual.

Fig 18. Micronuclei of *M. nova*.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